THE AMERICAN JOURNAL OF PHYSIOLOGY

VOLUME 141

BALTIMORE, MD.
1944

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THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 141 MARCH 1, 1944 No. 1

THE GLUCOSE NECESSARY TO MAINTAIN THE GLUCEMIA IN EVISCERATED DOGS

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Received for publication August 10, 1943

The study of carbohydrate changes in eviscerated animals as a method for investigating the glucose utilization in the peripheral tissues has been carried out by Soskin (1, 2) on the dog, by Mirsky and Broh-Kahn (3) on the rabbit, and by Russell (4) on the rat. We have carried out a large number of experiments in dogs in order to evaluate this method and to compare the results obtained in normal dogs with those obtained in hypophysectomized, diabetic and thyroid-treated dogs.

Experimental procedure. We have used dogs weighing between 13 and 23 kgm., most of which were males. The animals were allowed no food during the 18 hours preceding the experiment. They were anesthetized with chloralose at a concentration of 0.8 per cent in an 0.8 per cent NaCl solution. Normal controls were given 10 ml. per kilo of this solution intravenously, while hypophysectomized animals generally required 20 per cent less solution and thyroid-treated animals 20 per cent more. In this way a fairly uniform degree of anesthesia was obtained without interference with the glucose concentration of the blood.

The animals were eviscerated by the method described by Soskin and Mirsky (5), isolating the liver from the general circulation by the insertion of a glass cannula in the vena cava. Blood transfusions were given until the arterial blood pressure rose above 140 mm. Hg. Normal dogs were used as donors except for the diabetic animals where similarly diabetic donors were preferred. The animals were maintained under artificial respiration for the duration of the experiment. A standardized solution of glucose was delivered into the jugular vein at the rate of 1 ml. every 2 minutes, thus maintaining an almost continuous flow of glucose through the body.

In order to follow the changes in carbohydrate metabolism, blood glucose, blood lactic acid and muscle glycogen values were obtained. Blood obtained from the carotid artery was deproteinized after the method of Somogyi and glucemia determinations were made according to the method of Hagedorn and

[1] Canadian Federation of University Women's Travelling Fellowship.

Jensen. The glucemia was determined initially and every thirty minutes during the three-hour experimental period. The blood lactic acid was determined initially and at the end of the experiment by the method of Friedemann and Kendall (6) in the West aeration apparatus. Glycogen determinations were performed on the tibialis anticus, extensor digitorum longus and triceps surae muscles by the method of Pflüger; final titrations followed the method of Bertrand. One leg was used for the initial and the other for the final values.

We observed that the arterial blood pressure of eviscerated animals dropped to low levels. Glucose curves obtained at pressure levels below 50 mm. Hg gave erroneous values and were not taken into account. However, when the evisceration was performed in less than 20 minutes, and clotting in the vena cava was prevented by the use of a very clean dry glass cannula, and the animals were maintained on heated tables under artificial respiration, and adequate blood transfusions were given before beginning the glucose injection, then the blood pressure was maintained well above 50 mm. Hg. In this way blood sugar curves of 3 hours' duration, with a few of 2 hours' duration when the blood pressure was falling rapidly, were obtained. All experiments ending with pressure levels below 50 mm. Hg were discarded. By this criterion 78 out of 87 dogs were considered. The final pressure level was 110 to 80 mm. Hg in 13 cases, 60 to 80 mm. Hg in 52 cases and 50 mm. Hg in 13 cases. All animals were in relatively good shape with active reflexes at the end of the experiment.

The amount of glucose necessary to maintain the initial blood level was between 50 and 250 mgm. per kilo per hour. As shown in tables 1 and 2, various doses were tried on the following: 17 normal dogs, 14 hypophysectomized dogs, 15 dogs made diabetic through the injection of anterior pituitary extracts (7 ml. per kilo of a 20 per cent anterior pituitary extract given intraperitoneally, daily, during the 5 preceding days), 4 of which were previously thyroidectomized, 11 dogs pancreatectomized 24 hours before the experiment, and 11 dogs treated with desiccated bovine thyroid (0.5 gram per kilo per day administered by gastric tube during the 5 preceding days). The blood sugar values are expressed as the difference between the first and the last value of the curve. Positive or negative signs with ± 8 are considered as equal. Individual variations within the same dose level are great.

However, summarizing from the data in table 3, it is apparent that the amount of glucose needed to maintain the initial blood sugar in eviscerated dogs is as follows: In normal dogs, 100–200 mgm. per kilo per hour; in hypophysectomized dogs, 100–150 mgm. per kilo per hour; in hypophyseal diabetic dogs (thyroidectomy having no effect), 150–200 mgm. per kilo per hour; and in both pancreatic diabetic and thyroid-treated dogs, 150–200 mgm. per kilo per hour.

The blood lactic acid values, which initially varied between 8 and 14 mgm. per 100 ml. of blood, showed in almost all cases a progressive rise during the experiment, giving a positive difference of 10 to 50 mgm. per cent between initial and final values (tables 1 and 2). The difference between initial and final muscle glycogen values is very variable. Most of the initial values were between 400 and 600 mgm. per cent.

DISCUSSION. We have observed that when the arterial blood pressure is maintained below 50 mm. Hg, there is a marked reduction in the sugar consumption, probably due to the consequent state of anoxia. However, eliminating all animals in which the blood pressure fell to such low levels, we were able to obtain regularly rising or falling blood sugar curves which permitted us to establish the approximate amount of glucose necessary to maintain the glucemia at its initial

TABLE 1

Carbohydrate balance of eviscerated dogs receiving continuous glucose injection

GLUCOSE INJECTED	NORMAL				HYPOPHYSECTOMIZED			
	Glucemia (mgm. %)		Lactic acid (mgm. %) difference	Glycogen (mgm. %) Difference	Glucemia (mgm. %)		Lactic acid (mgm. %) difference	Glycogen (mgm. %) difference
	Difference	Initial Final			Difference	Initial Final		
mgm./kgm./hr.								
50	−63	(94– 31)	+26	+108	−45	(90– 45)	+26	−115
50	−52	(92– 40)	+26	+109	−27	(95– 68)	−1	−172
50	−15	(87– 72)	+28	+10				
100	+9	(41– 50)	+30	+133	+93	(71–164)	+7	+197
100	=4	(85– 81)	+51	+162	−25	(97– 72)	+7	−103
100	=4	(94– 98)	+6	+119	=6	(103– 97)	+23	−127
100					+9	(78– 87)	+5	−116
100					+11	(83– 94)	+12	−460
150	−20	(77– 57)	+16	−285	+61	(76–137)	+52	−80
150	=4	(46– 50)	+8	−59	=2	(68– 66)	+39	−3
150	=0	(102–102)	+23	−57	+16	(78– 94)	+46	−200
150					+22	(90–112)	+83	−105
150					=4	(109–105)	+14	−67
200	+46	(74–119)	+64	+63	+11	(66– 77)	+25	−150
200	=0	(96– 96)	+3	+43				
200	−9	(103– 94)	+24	−136				
200	=2	(80– 82)	+19	+64				
200	+16	(78– 94)	+15	+25				
250	+54	(60–114)	+73	+98				
250	+118	(94–212)	+28	−70				
250	+62	(75–148)	−5	−124				

level, in eviscerated animals. Our results, however, when calculated as sugar utilization on the basis of Soskin's calculations, (1) did not give consistent values; in fact, we found these calculations of no possible use in our experiments.

Therefore, we have based our conclusions mainly on the blood sugar curve, referring to the amount of glucose in milligrams per kilo per hour necessary to maintain the initial blood sugar level.

Individual variations were very great making it impossible to arrive at exact

doses. However, disregarding certain isolated cases, the results on a large series of animals indicate that eviscerated normal dogs require from 100–200 mgm. of

TABLE 2

Carbohydrate balance of eviscerated dogs receiving continuous glucose injections

GLUCOSE INJECTED	DIABETIC (A.L.H.)					DIABETIC (PANCREATIC)					THYROID-TREATED				
	Glucemia (mgm. %)			Lactic acid (mgm. %)	Glycogen (mgm. %)	Glucemia (mgm. %)			Lactic acid (mgm. %)	Glycogen (mgm. %)	Glucemia (mgm. %)			Lactic acid (mgm. %)	Glycogen (mgm. %)
	Difference	Initial	Final	difference	difference	Difference	Initial	Final	difference	difference	Difference	Initial	Final	difference	difference
mgm./kgm./hr.															
100	−12	(134	122)	+45	−200						−35	(100	65)	+23	−26
100	+9	(134	143)	+20	−105						+31	(91	122)	+24	−20
100	−40	(259	219)	+5	−8						−9	(76	67)	+22	−190
150	−271	(203	131)	+40		−80	(274	194)	+47	−110	−0	(112	112)	+57	−220
150	=2	(129	127)	+50	−94	−42	(272	230)	+15	−50	−34	(121	97)	+44	−127
150	−68	(205	151)	+4	−365	−22	(216	184)	+10	−10	−12	(91	79)	+34	+154
150	+25	(133	158)	+10	−33										
150	+83	(182	268)	+2	−80										
200	=5*	(144	149)	+20		+40	(244	284)	+7	−150	+81	(83	164)	+40	−24
200	+19*	(170	189)	+19	+120	+124	(199	323)	−13	−114	+51	(103	154)	+68	−82
200	=4*	(157	161)	+30	+160	−53	(283	230)	+17	+10	=3	(68	65)	+18	+17
200	−22	(190	178)	+6	+68	+46	(222	268)	+8	+64	+18	(96	114)	+27	+172
200	+67	(145	212)	−19	−28	+87	(144	231)	+6	+37	+42	(91	133)	+25	+14
200	=6	(265	259)	+20	−35										
200	=4	(183	187)	+72	+130										
200	−34	(158	124)	+42	−17										
250	+60	(189	249)	+34	−324	+61	(242	303)	+57	−9					
250	+28	(162	190)	+40	+102	+105	(231	336)	+14	−90					
250	+40	(136	176)	+16	−110	+78	(170	248)	+11	−10					

* Thyroidectomized.

TABLE 3

Difference between initial and final glucemia in eviscerated dogs with continuous glucose injection

GLUCOSE INJECTED	NORMAL			HYPOPHYSECTOMIZED			DIABETIC (A.L.H.)			DIABETIC (PANCREAT.)			THYROID-TREATED		
	−	=	+	−	=	+	−	=	+	−	=	+	−	=	+
mgm./kgm./hr.															
50	3			2											
100	1	2		1	1	3	2		1				2		1
150	1	2			2	3	2	1	2	3			2	1	
200	1	2	2			1	2	4	2	1		4		1	4
250			3						3			3			

−, fall of blood sugar; =, maintenance of blood sugar (± 8 mgm. %); +, rise of blood sugar.

glucose per kilo per hour to maintain their initial glucemia level. These results are in accordance with those of other investigators (10). In the hepatectomized

dog, Mann (11) observed that 250 mgm. of glucose per kilo per hour were required in animals which were not anesthetized, but were kept quiet and warm. He also calls attention to the wide individual variation.

Hypophysectomized eviscerated dogs required 100–150 mgm. of glucose per kilo per hour to maintain their glucemia level. This dose is within the range of that for normal dogs. It is worth mentioning that the blood pressure in hypophysectomized dogs tends to fall more rapidly than in other cases. Soskin et al. (7) observed that hypophysectomized dogs gave lower than normal sugar utilization values. In the five dogs studied they report a consumption subnormal even to the level for pancreatectomized animals. These same authors noted that glycogen is very stable, which fact we could not confirm.

In non-eviscerated hypophysectomized dogs Chambers et al. (8) did not observe an increased sugar consumption. The rabbit and rat, on the other hand, seem to need very high sugar levels, as observed by Greeley (9). He used 700 mgm. of glucose in the hypophysectomized rabbit to prevent hypoglycemic levels upon fasting. Russell (4) reported that 250 mgm. of glucose were needed to maintain the glucemia in hypophysectomized and eviscerated rats as compared to 135 mgm. of glucose needed in the eviscerated controls.

The results obtained in pancreatectomized dogs were more uniform and these animals required a maintenance dose of 150–200 mgm. of glucose per kilo per hour. Animals made diabetic through the injection of anterior pituitary extract required from 150–200 mgm. per kilo per hour. Previous thyroidectomy in 4 of these dogs in no way affected the results. The amount of glucose needed to maintain the high initial blood level of a diabetic dog is within the range of the normal, although within the higher values. Soskin (2) observed that anterior pituitary extract does not influence the rate of sugar utilization by the extrahepatic tissues of normal dogs. Soskin (1, 2) also maintains that pancreatectomized animals utilize less sugar at a given glycemia level than do normal dogs at the same level. Mirsky and Broh-Kahn (3) observed an increased peripheral utilization of glucose in the thyroid-treated rabbit.

In our experiments it was found that eviscerated thyroid-treated dogs, just as pancreatectomized animals, required 150–200 mgm. glucose per kilo per hour to maintain the initial glycemia level. This is within the normal range, although within the higher values.

CONCLUSIONS

1. Whether the eviscerated dog can be regarded as normal except for the deprivation of liver and viscera is a questionable hypothesis.

2. Calculation of the carbohydrate balance from the data obtained in our experiments on eviscerated dogs gave improbable results.

3. When the blood sugar curve alone is considered, in the absence of hypotension or asphyxia, it is possible to determine the amount of glucose that must be injected in order to maintain the initial glucemia level. Although individual variations are great, studies on a large number of animals reveal that the maintenance dose of glucose is 100–200 mgm. per kilo per hour for normal dogs, 100–150 mgm. per kilo per hour for hypophysectomized dogs, 150–200 mgm. per kilo

per hour for dogs made diabetic by the injection of anterior pituitary extract, and 150–200 mgm. per kilo per hour in both pancreatectomized and thyroid treated dogs.

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THE EFFECT OF SODIUM 5,5-DIPHENYL HYDANTOINATE (DILANTIN SODIUM) UPON THE TOLERANCE OF RATS AND MICE TO DECOMPRESSION[1]

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Received for publication October 27, 1943

Convulsive seizures constitute a striking feature of the late stages in the reaction of experimental animals to severe acute anoxia (1). In view of the successful use of dilantin as an anticonvulsant in epilepsy (2), the experiments to be described in this report were undertaken to test whether this drug would delay or prevent experimental decompression convulsions and whether its administration would significantly affect the tolerance of animals to the conditions prevailing at reduced atmospheric pressures.

METHODS. The results to be reported are based on a study of 118 rats and 159 mice, grouped into three series. Series I comprised 77 young adult rats, weighing approximately 200 grams each, which were decompressed individually in a glass decompression chamber, without added oxygen but with adequate ventilation, at a uniform rate of 100 mm. Hg per minute. Each animal was at first given a control decompression to establish its "altitude ceiling". Decompression was continued to the pressure at which breathing stopped and at this point the animal was instantaneously recompressed. From one to eight days after this control "flight" the surviving animals were again each subjected to a period of progressive lowering of atmospheric pressure. Three to four hours before this second decompression the rat was injected subcutaneously with an aqueous suspension of sodium 5,5-diphenyl hydantoinate (Kapseals Dilantin Sodium; Parke, Davis & Company). Decompression was carried out at the same rate as in the control "flight" to the point of apnea. A new "altitude ceiling" having been determined, the animal was again immediately returned to normal room pressure.

In series II there were 41 rats. The object of the experiments in this series was to determine the length of time of survival of control animals at a given simulated altitude and to compare these survival times with the performance of a second group of animals from the same colony, which received sodium 5,5-diphenyl hydantoinate before decompression.

In a preliminary group of three control animals, times of survival at 150, 100 and 70 mm. Hg were recorded. Twenty experiments were then carried out in which normal control rats were decompressed, at the standard rate of 100 mm.

[1] These studies were supported by a grant from the Trustees of Sarah Lawrence College. A preliminary report appeared in the Federation Proceedings 2: 22, 1943.

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Hg per minute, to 70 mm. Hg at which pressure all animals were still breathing. In each experiment, the animal's time of survival at this "altitude" was recorded with precision. In this control group, each animal was recompressed approximately thirty seconds after breathing stopped. All but two controls were dead on recompression. In 18 rats, the effect of dilantin on survival time at 70 mm. Hg was investigated. The drug was given subcutaneously three or four hours before decompression, and in every case the animal was taken to 70 mm. Hg at the standard rate. The pressure was maintained at this level until breathing stopped; the time was recorded and the animal recompressed.

In series III a group of experiments similar in pattern to those of series II was conducted on 159 young adult mice weighing approximately 15 grams. Animals were decompressed individually at the rate of 100 mm. Hg per minute to 150 mm. Hg, a pressure level found in preliminary runs to be lethal to control subjects within less than 2 minutes. The survival time at this pressure was determined in 73 control mice, all but six of which failed to resume respiration on return to the laboratory barometric pressure. Survival times were also recorded in 73 mice from the same colony and of similar age and weight, subjected to decompression within 10 minutes to 48 hours after intraperitoneal injection of 0.5 to 3.0 mgm. of dilantin (Dilantin Sodium in Glaseptic Ampoules, for intravenous use, no. 3239005)[3] in a 0.9 per cent saline solution containing 10 mgm. of the drug per cubic centimeter. Throughout this series the control runs were interspersed between decompression experiments on the drugged animals and all control animals were injected intraperitoneally with an amount of 0.9 per cent saline solution equal in volume to the drug solution received by the dilantinized mice decompressed during the same experimental session, which consisted usually of 12 control decompressions and 12 drug runs.

Results. *Series I. Effect of subcutaneous administration of dilantin upon the pressure "ceiling" attained by rats.* After premedication with dilantin rats tolerated a lower pressure than was possible during their control decompression. In the first "flights", carried out without the drug, the animals exhibited the usual signs associated with rapid ascent to high altitudes, commencing with heightened motor excitability, muscular inco-ordination and characteristic chewing movements. At the lower pressures, there was enlargement of the abdomen, gross inco-ordination, opisthotonus and convulsive episodes succeeded by coma, apnea and, in some cases, cardiac arrest.

Twenty-eight rats died during control decompression. The "ceiling" pressures reached by these animals varied from 95 to 17 mm. Hg, an average of 42 mm. Hg. As shown in table 1, twenty-five animals which survived the control "flights" as well as decompression after drug administration attained *control* "ceilings" ranging from 55 to 17 mm. Hg, an average of 35 mm. Hg. Subcutaneous injection of dilantin in doses used in these experiments resulted in marked slowing of the respiration, and no rat was subjected to decompression until the respiratory rate had fallen below 70 per minute. Under influence of

[3] We wish to express our thanks to the Research Department of Parke, Davis & Company for a generous supply of this preparation.

the drug, the animals tended to lie quietly when undisturbed, but exhibited greatly exaggerated motor response to tactile stimuli. During decompressions, the drugged animals were less active; opisthotonus was not so marked, but the degree of abdominal distention was the same as in the control "flights". As table 1 shows, the "ceilings" attained by animals after medication ranged from 37 to 5 mm. Hg, an average of 18 mm. Hg. In all but one case (expt. 5), the animals tolerated a lower pressure with the drug than without. It may also be pointed out that the pressure levels successfully attained by the dilantinized animals were, on the average, higher than the "ceilings" reached by the group of 28 animals which failed to survive the control decompression. Eleven of the controls which died succumbed at pressures within the range of the "ceilings" of the medicated animals.

If the animals reported in table 1 are listed in order of increasing initial "altitude" tolerance, it can be seen that dilantin has a relatively greater effect in raising the resistance of animals with a lower control "ceiling" than those with higher initial tolerance to decompression. Under the conditions of these experiments in which a fairly uniform group of rats was used, there was no significant correlation between body weight and capacity to withstand low pressures.

Eight rats failed to survive the second decompression although they had received injections of dilantin. Attention is drawn, however, to the fact, illustrated in table 2, that all of these animals reached significantly higher "ceilings" after drug administration than during their control "flights". Sixteen rats surviving the control "flight" were not decompressed under the influence of the drug. Of these, 7 died, presumably from an overdose of dilantin. The remaining 9 were not used because dilantin failed to reduce the respiratory rate to less than 70 per minute.

Series II. Effect of subcutaneous injection of dilantin upon the survival time of rats at a pressure of 70 mm. Hg. Experiments carried out on three control animals maintained at different pressure "ceilings" yielded the following data: Rat 7a was decompressed to 150 mm. Hg and held at this pressure for 5 min. 15 sec.; respiration continued and the animal survived after recompression. Rat 6a died after 2 min. 30 sec. at 100 mm. Hg while rat 5a remained alive at 70 mm. Hg for 1 min. 30 sec. and was recompressed dead. The pressure requiring a shorter lethal period, *i.e.*, 70 mm. Hg was selected as a convenient "ceiling" at which to maintain rats for the study of survival times.

Twenty control rats decompressed at the standard rate to 70 mm. Hg were all dead after an average exposure of 1 min. 30 sec. at "ceiling" pressure. The longest time tolerated was 1 min. 50 sec., the minimum time for a lethal effect being 1 min. 15 sec.

Eighteen rats were decompressed after subcutaneous injection of dilantin. The results of these experiments are given in table 3. All of these animals survived longer than any in the control group. Rat 12b stopped breathing after 10 min. 10 sec. exposure, and was recompressed dead. Rat 25b was alive after an exposure of 10 min. 55 sec. and was decompressed once again during the same afternoon. At the second decompression, the animal was still breathing after 4

TABLE 1

"Ceilings" of rats surviving control decompression as well as decompression after subcutaneous administration of dilantin

EXPT. NO.	WEIGHT	CONTROL "CEILING"	INTERVAL BETWEEN CONTROL AND DRUG EXPTS.	DOSE	DRUG "CEILING"
	grams	*mm. Hg*	*days*	*grams*	*mm. Hg*
4	238.2	45	1	0.2	37
5	214.7	25	1	0.4	27
6	239.3	35	4	0.3	23
9	370.0	30	1	0.4	19
10	139.2	30	8	0.3	17
11	142.6	25	1	0.3	12
12	343.0	55	1	0.3	25
14	207.4	47	1	0.3	17
15	178.7	55	1	0.3	29
18	192.0	45	1	0.3	20
21	191.1	36	1	0.3	17
24	227.8	40	2	0.3	17
28	290.0	25	1	0.3	23
36	338.4	48	1	0.3	19
38	88.1	44	1	0.2	27
42	108.9	54	1	0.3	28
43	197.8	27	1	0.4	9
49	370.0	17	1	0.3	5
59	186.4	38	1	0.3	28
66	173.6	40	1	0.3	25
71	176.1	20	1	0.3	9
72	202.0	20	1	0.3	5
73	132.9	20	1	0.3	11
76	167.6	20	1	0.3	10
77	186.3	25	1	0.3	6

TABLE 2

"Ceilings" of rats which died during the second decompression (after subcutaneous administration of dilantin)

EXPT. NO.	WEIGHT	CONTROL "CEILING"	INTERVAL BETWEEN CONTROL AND DRUG EXPTS.	DOSE	DRUG "CEILING"
	grams	*mm. Hg*	*days*	*grams*	*mm. Hg*
2	187.7	70	1	0.1	27
3	311.0	80	1	0.1	27
8	180.7	25	1	0.3	5
23	242.8	30	2	0.3	9
34	156.4	54	1	0.2	30
44	240.0	32	1	0.3	7
51	290.0	69	1	0.3	20
56	217.7	32	1	0.3	21

min. and survived the experience. The minimum exposure necessary to kill any of the drugged rats was 3 min. 8 sec. (expt. 16b) and the average time was 4 min. 47 sec.

Series III. The effect of intraperitoneal injection of dilantin upon the survival time of mice at a pressure of 150 mm. Hg. During decompression under the conditions of these experiments control mice presented a very uniform picture. In each case, as the pressure fell, the animal became restless and active and usually continued to explore the wire cage within which it was confined until a level of about 250 to 200 mm. Hg was reached. During this period there was a progres-

TABLE 3

Time tolerated at 70 mm. Hg by rats after subcutaneous administration of dilantin (controls killed within 1 min. 50 sec.)

EXPT NO.	WEIGHT	DOSE	TIME TO APNEA		TOTAL TIME AT "CEILING"		FATE OF ANIMAL
			Min.	Sec.	Min.	Sec.	
	grams	*grams*					
11b	188.4	0.3	3	15	3	25	Died
12b	187.0	0.4	10	10	10	35	Died
13b	177.9	0.3	3	30	4	15	Died
14b	263.7	0.3	6	15	6	45	Died
15b	214.4	0.3	9	5	9	5	Lived 1 min.
16b		0.3	2	15	3	8	Died
17b	232.4	0.3			3	40	Lived 10 min.*
18b	206.3	0.4			3	25	Lived 9 min.*
19b	190.0	0.4	4	0	4	10	Died
20b	213.9	0.5	4	34	5	0	Died
21b	200.3	0.3	3	45	3	45	Died
22b	238.0	0.4	4	10	4	25	Died
23b	187.0	0.4	3	15	3	35	Died
24b	200.0	0.3			4	7	Recovered*
25b	216.9	0.3			10	55	Recovered*
25b	216.9	Same			4	0	Recovered*
26b	200.3	0.4			3	15	Lived 2 min.*
27b	252.0	0.4	4	25	4	25	Died
28b	208.0	0.4	3	25	3	25	Died

*Experiments in which apnea did not supervene.

sive acceleration of respiration and increasing cyanosis. With further drop in pressure, the mouse tended to sit quietly huddled up or to move about with a slow ataxic gait. Ordinarily, however, motor function was not gravely disturbed until the pressure had been at 150 mm. Hg for approximately 30 sec. to 1 min. when, with dramatic suddenness, the animal was seized with convulsions. These attacks were characterized by protrusion of the eyeballs, opisthotonus and spasmodic movements of the extremities, particularly of the hind limbs which were rigidly extended backwards. The animal kicked convulsively, often throwing itself the entire length of the cage. There was also spasmodic twitching of the tail which was arched dorsally. The eyes were black and the snout, paws and

tail deeply cyanotic. Within a few seconds respirations became slow and gasping and shortly ceased altogether. Although most control animals tolerated the "ceiling" pressure for 30 to 60 sec. before the onset of the seizures, many did not breathe at all after the "ceiling" had been attained and died quickly after a few violent extensor spasms. Considerable variability in the severity of the attacks was observed and in some instances breathing became gradually slower and weaker and ceased without any convulsive episode. There was no relation between the length of the survival period and the occurrence or non-occurrence of the seizures or their violence. The average time required to kill 67 control mice at "ceiling" pressure was 1 min. 4 sec., the most resistant animal dying after an exposure of 3 min. 15 sec. and the shortest intervals sufficient to destroy life at 150 mm. Hg being 2 and 3 sec. In all the other fatal cases, the time intervals ranged between 10 sec. and 1 min. 58 sec. The six control animals which recovered after recompression were kept at 150 mm. Hg for 59 sec. to 1 min. 29 sec. In each of these cases, the chamber was recompressed the instant breathing ceased whereas in all of the other control mice the "ceiling" pressure was held for approximately 20 to 30 sec. after apnea.

Intraperitoneal injection of 0.5 mgm. of dilantin resulted in no obvious disturbances in mice. However, within 18 min. after receiving 1.0 mgm. animals walked with the limbs slightly splayed outwards and within an hour exhibited ataxic gait but quickly righted themselves if turned on their backs. This unsteadiness persisted for as long as 24 hrs. but the animals remained otherwise in good condition. Doses of 2.0 and 3.0 mgm. produced similar effects but ataxia was more pronounced and there were slow side to side tremors of the head. In a group of 10 trial mice it was found that intraperitoneal doses of 4.0 mgm. or more resulted in death in less than 48 hrs. Within 15 min., such animals were markedly ataxic. They became progressively weaker and within 30 min. to 1 hr. remained on their backs or in any position in which placed. Periodic spasmodic twitching movements were observed especially when the animals were touched.

Twenty-six mice decompressed within 10 min. to 48 hrs., 12 min. after intraperitoneal injection of 2.0 mgm. of dilantin all tolerated longer periods at "ceiling" pressure than the corresponding controls. As shown in table 4, the first 12 of these drugged animals continued to breathe at 150 mm. Hg for 6 min. 0 sec. to 10 min. 10 sec., an average of 7 min. 53 sec., whereas an average period of 1 min. 1 sec. was lethal to the controls decompressed during the same day and all fatalities in the control group occurred within 1 min. 30 sec. Not only did the drugged mice tolerate longer times at the "ceiling", but also all but two recovered on recompression. In these animals, the interval between injection of dilantin and the onset of decompression varied from 8 hrs., 30 min. to 11 hrs., 44 min. (average, 10 hrs., 14 min.). As seen in table 5, however, dilantin exerted an appreciable protective action as early as 10 min. after injection of 2.0 mgm. and in experiment 146c, commenced 48 hrs., 12 sec. after administration of the drug, the animal was still breathing after 22 min. 30 sec. at 150 mm. Hg. In the group of 14 drugged animals listed in this table, the time at "ceiling" pressure

TABLE 4

Time tolerated at 150 mm. Hg by mice receiving 2.0 mgm. dilantin intraperitoneally (controls killed within 1 min. 30 sec.)

EXPT. NO.	WEIGHT	INTERVAL BETWEEN INJECTION AND DECOMPRESSION		TIME AT "CEILING"		FATE OF ANIMAL	OBSERVATIONS
		Hrs.	Min.	Min.	Sec.		
	grams						
63c	15.8	8	30	7	15	Recovered	No convulsions
64c	14.1	8	50	7	03	Recovered	Running movements
65c	14.1	9	12	8	15	Recovered	Running movements
66c	15.3	9	32	8	20	Died	No convulsions
67c	11.8	9	48	10	10	Recovered	Some terminal spasms
68c	17.5	10	5	7	45	Delayed death	No convulsions
69c	15.9	10	32	7	40	Recovered	Few terminal spasms
70c	13.4	10	49	7	35	Recovered	Slight terminal seizure
71c	13.5	11	8	6	22	Recovered	No convulsions
72c	17.0	11	10	9	30	Recovered	No convulsions
73c	16.1	11	30	6	0	Recovered	No convulsions
74c	13.5	11	44	8	45	Recovered	Slight terminal seizure

TABLE 5

Time tolerated at 150 mm. Hg, illustrating the duration of the protective effect exerted on mice by 2 mgm. dilantin administered intraperitoneally (controls killed within 3 min. 15 sec.)

EXPT. NO.	WEIGHT	INTERVAL BETWEEN INJECTION AND DECOMPRESSION		TIME AT "CEILING" (150 MM. Hg)		FATE OF ANIMAL	OBSERVATIONS
		Hrs.	Min.	Min.	Sec.		
	grams						
147c	10.0	0	10	4	5	Died	No convulsions
148c	10.3	0	30	26	0	Recovered*	No convulsions
149c	10.6	0	35	8	30	Died	No convulsions
135c	18.6	1	20	7	15	Delayed death	Some terminal spasms
136c	12.3	2	0	20	15	Recovered*	No convulsions
137c	10.7	3	49	60	0	Recovered*	No convulsions
138c	11.0	6	44	19	0	Recovered*	No convulsions
139c	11.3	7	29	21	0	Delayed death	Some terminal spasms
141c	11.4	8	30	19	40	Delayed death	Terminal extensor spasms
142c	14.7	10	33	32	0	Recovered*	No convulsions
143c	10.0	18	27	4	20	Died	No convulsions
144c	12.3	18	39	30	0	Recovered*	No convulsions
145c	10.8	32	4	61	30	Recovered*	No convulsions
146c	12.0	48	12	22	30	Recovered*	No convulsions

* Experiments in which apnea did not supervene.

varied from 4 min. 5 sec. to 61 min. 30 sec. (average, 24 min. 0 sec.). Eight of these animals (indicated by an asterisk) continued breathing throughout the experiment and could undoubtedly have tolerated an even longer exposure. All

eight survived. Three other mice in this group (expts. 135c, 139c, 141c) breathed on recompression but died subsequently. In the remaining three, respirations failed to return. All control animals corresponding to this group died in convulsions after average exposure of 1 min., 35 sec. at 150 mm. Hg, the longest time being 3 min. 15 sec. and the minimum lethal period being 1 min. 0 sec.

Animals receiving 2.0 mgm. of dilantin were usually somewhat quieter during decompression than were the controls. Deep cyanosis was as prominent a feature as in the control group. There was frequently blinking of the eyes with exophthalmos, and all dilantinized mice surviving these prolonged periods developed marked corneal opacity which gradually disappeared within 45 min. after the end of the run. In all mice injected with 2.0 mgm. of the drug, decompression convulsions were either absent or considerably delayed. Where they occurred at all, the seizures were usually represented by spasmodic walking movements of the limbs, periodic twitches in the trunk or moderate retraction of the head. In no instance were any convulsive movements observed on recompression.

In 23 mice decompressed within 5 hrs., 31 min. to 11 hrs., 29 min. after intraperitoneal injection of 3.0 mgm. of dilantin the survival times were also longer than in corresponding control animals (although, in general, not as long as in animals receiving 2.0 mgm.) the shortest period being 2 min. 0 sec. and the longest, 13 min. 40 sec. (average, 4 min. 53 sec.). Ten of these animals recovered; 13 failed to breathe after recompression. All of the controls decompressed in the same experimental sessions were killed by periods from 2 sec. to 2 min. 0 sec. (average, 46 sec.). Motor phenomena observed in this group during decompression were similar to those seen after 2.0 mgm. injections. Two animals succumbed approximately 9 hrs. after drug administration and were therefore not subjected to decompression.

Intraperitoneal injection of 1.0 mgm. of dilantin afforded some protection in 12 mice decompressed 6 hrs., 55 min. to 9 hrs. 4 min. later. Time at 150 mm. Hg ranged from 1 min. 0 sec. to 11 min. 37 sec. (average, 4 min. 40 sec.) while 11 out of 12 controls were all dead after periods from 50 sec. to 1 min. 25 sec. (average 1 min. 9 sec.). In the drugged animals of this group terminal convulsive episodes were observed which closely resembled in frequency and severity those seen in the controls but whereas all but one control animal failed to breathe on recompression, 7 of these 12 dilantinized mice recovered. Doses of 0.5 mgm. appeared to have little or no effect on the tolerance of mice to the "ceiling" pressure. Twelve mice decompressed from 5 hrs. 25 min. to 8 hrs. 25 min. after intraperitoneal injection of 0.5 mgm. of dilantin, died in convulsions after 55 sec. to 3 min. 5 sec. (average, 1 min. 43 sec.) at 150 mm. Hg. A control group of 12 mice were all killed by periods ranging from 30 sec. to 1 min. 45 sec. (average, 1 min. 4 sec.).

Discussion. Under the conditions of these experiments in which decompression was carried out at the rate of 100 mm. Hg per minute, rats and mice given single doses of dilantin tolerated lower pressures or withstood a given low pressure for a longer time than without the drug. Using apnea as an end point,

dilantinized animals can thus be taken to extraordinarily low pressures and survive. It is not pretended, however, that such pressures are compatible with life for more than a brief period even with the protection of the drug. In determining the effect of dilantin upon the survival time of rats and mice, very low pressures (70 mm. Hg and 150 mm. Hg) were purposely chosen such that control animals succumbed within about a minute or two. No data are yet available as to the possible protective action of dilantin in animals maintained at somewhat higher pressure levels than those here reported or decompressed at slower rates. Nor has the effect of this drug been investigated in decompression experiments such as those of Büchner and Luft (3) in which animals are subjected to reduced pressures continuously over a period of several days.

The doses of dilantin given to rats in series I and II are large. In these animals, the form of the drug used was that intended for oral administration and containing a lactose diluent. Very possibly absorption of dilantin from subcutaneously injected sites under these circumstances was not complete at the time of decompression. It is noted, however, that seven rats died after subcutaneous injections of the oral preparation. Using dilantin prepared for intravenous administration, the doses required were very much less. Intraperitoneal injection of as little as 1.0 mgm. prolonged the period tolerated at 150 mm. Hg by 10 to 15 gram mice while the optimal dosage was 2.0 mgm. At this dose, dilantin increased the resistance of mice decompressed within 10 min. or as late as 48 hours after injection. As illustrated in tables 3 and 5, the protection conferred by dilantin in terms of increase in time tolerated at a given "ceiling" pressure was greater in mice receiving 2.0 mgm. than in rats subcutaneously injected with 0.3 to 0.4 gram of the oral preparation. The data do not, however, permit any conclusion as to a possible species difference in the protective action of dilantin in rats and mice.

While all drugged animals supported a given "ceiling" pressure for a longer time than corresponding controls, a greater individual variation was observed in the times tolerated by the former than by the latter. In neither species was there any relation between the sex of the animals and either their tolerance to control decompression or the increase in resistance afforded by dilantin. Side effects such as tremor and ataxia were observed in mice following all doses of dilantin effective in increasing resistance to decompression. Doses of 2.0 and 3.0 mgm. not only prolonged the interval tolerated by mice at "ceiling" pressure but also prevented or mitigated and delayed convulsive episodes. Mice receiving 1.0 mgm. of the drug tolerated a longer average interval and showed a higher survival rate than control animals although the incidence and severity of terminal seizures were unaffected.

No conclusion can at present be drawn as to the mechanism of the protective action of dilantin in acute decompression. It may be tentatively proposed that the drug prolongs normal function of nerve cells, in particular of the respiratory and cardiac centers, under conditions of severe oxygen lack. The possibility that dilantin may cause a generalized lowering of basal metabolic rate is under investigation. Experiments now in progress indicate that dilantin also increases

resistance to the lethal effects of anoxia induced by means other than decompression. Since severe and closely similar neuronal lesions are found in death from decompression (1, 3), low oxygen mixtures (4), carbon monoxide (5), and nitrous oxide (6), as well as from hypoglycemia (7) and electric shock (8), studies are now being carried out to determine whether such pathological changes may be prevented or their incidence effectively reduced by administration of dilantin.

SUMMARY

1. A study of the effect of sodium 5,5-diphenyl hydantoinate (Dilantin Sodium; Parke, Davis & Company) upon tolerance to reduced atmospheric pressures has been carried out on 118 rats and 159 mice.

2. After subcutaneous injection of dilantin, rats sustained a lower pressure than was possible during their control decompression. Animals surviving both a control "flight", as well as decompression after drug administration, attained an average control "ceiling" of 35 mm. Hg, while dilantin administration enabled them to reach "ceilings" from 37 to 5 mm. Hg, an average of 18 mm. Hg. The "ceiling" was taken as the pressure at which breathing ceased.

3. Dilantin had a relatively greater effect in raising the tolerance of rats with a lower control resistance than those with greater initial tolerance to decompression.

4. Control rats decompressed at a standard rate of 100 mm. Hg per min. to 70 mm. Hg all died after an average exposure of 1 min. 30 sec. at this "ceiling" pressure, the longest exposure for a lethal effect being 1 min. 50 sec. Rats decompressed under these conditions after subcutaneous injection of dilantin survived from 3 min. 8 sec. to 10 min. 55 sec., the average lethal exposure being 4 min. 47 sec.

5. Intraperitoneal injection of 1.0, 2.0 or 3.0 mgm. of dilantin in every case prolonged the time tolerated by mice to a pressure of 150 mm. Hg. Seventy-six control animals were all killed by exposure to this pressure for 2 sec. to 3 min. 15 sec. (average 1 min. 4 sec.), whereas with 2.0 mgm. doses of dilantin mice continued to breathe at 150 mm. Hg for 4 min. 5 sec. to 61 min. 30 sec. (average, 15 min. 56 sec.). The protective action of the drug could be demonstrated within 10 min. and persisted as long as 48 hrs. after injection.

6. In both rats and mice, dilantin abolished or delayed decompression convulsions. However, with doses as low as 1.0 mgm., convulsive episodes occurred in mice although survival time was prolonged.

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THE EFFECT OF CARBON MONOXIDE ON THE OXYHEMOGLOBIN DISSOCIATION CURVE

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Received for publication October 29, 1943

J. B. S. Haldane (13) was the first to study theoretically the equilibrium between hemoglobin and mixtures of O_2 and CO which were of insufficient concentration to saturate the hemoglobin completely. His treatment was subsequently amplified and modified by A. V. Hill (15), Stadie and Martin (23), Barcroft (4), and Peters and Van Slyke (19). As a result of this work it has been generally accepted that partial saturation of the blood hemoglobin with CO shifts the oxygen dissociation curve of the remaining hemoglobin progressively to the left, and also makes the curve less S-shaped and more hyperbolic[1] ("Haldane effect"). This indicates that the blood in vivo, if partially saturated with CO, must cling to its O_2 with greater tenacity so that the tissues have much more difficulty in obtaining O_2 from the blood than they do when the O_2Hb of the blood is reduced to a corresponding extent by anemia. This deduction has been used by Haldane to explain why "Miners may do their ordinary work though their hemoglobin percentage is reduced to half by ankylostomiasis—whereas a person whose blood is half saturated with CO is practically helpless."

A somewhat puzzling and paradoxical feature of CO-poisoning which has been explained by the Haldane effect is that with mice at very low pO_2 the addition of a trace of CO to the inspired air may actually help the organism to acquire O_2.

Haldane's original theory was only tested experimentally by observations on the CO-dissociation curve of hemoglobin when part of the hemoglobin was combined with O_2; parallel observations as to the effect of partial combination of the hemoglobin with CO on the O_2 dissociation curve of the remaining hemoglobin were not available until the work of Stadie and Martin twelve years later. These authors considered that their hypothesis was well supported by what they thought was excellent agreement between theory and experiment. Doctor Stadie in a recent conversation has, however, permitted us to say that in his present opinion the agreement was not as good as could be desired, particularly as the observations were limited to a somewhat narrow portion of the dissociation curve.

Since the subject is of interest, alike in the theory of the hemoglobin reactions and in the practical study of the effects of CO poisoning both at sea level and high altitudes, we have thought it desirable to reinvestigate the subject in a more complete fashion. In so doing we have been led to some profitable simplifications in the development of the theory. It is satisfactory to find that the original

[1] Partial saturation of the hemoglobin with O_2 has an exactly similar effect on the CO-dissociation curve of the residual hemoglobin.

Haldane theory, as thus extended, has stood up well to our more searching tests and we feel that it can therefore be used with confidence in more detailed calculations as to the toxicity of CO especially at high altitudes. The latter problem has been considered, as regards its importance in aviation medicine, by Heim (14).

Review of the Haldane theory and description of simplified method of application. When blood or hemoglobin is brought into equilibrium with gas mixtures containing O_2 and CO at pressures such that the amount of reduced hemoglobin is negligible, the proportion of CO-hemoglobin to O_2-hemoglobin is generally agreed to follow the equation:

$$\frac{[\mathrm{COHb}]}{[\mathrm{O_2Hb}]} = \frac{\mathrm{MpCO}}{\mathrm{pO_2}} \tag{1}$$

The value of M is independent of pH, salts and dilution of the blood, but varies with temperature, illumination and species. There is some dispute as to whether M varies from individual to individual in a given species; thus Douglas, Haldane and Haldane (11) with their carmine titration method of estimating COHb, found values of M ranging from 220 to 290 in man, whilst Killick (17) with the reversion spectroscope reported figures of 230 to 270 in man. Sendroy, Liu and Van Slyke (22), however, claim that for six men M is constant at 210 to ±2.5 per cent, and for ten different ox bloods is constant at 179 (±2.5 per cent). These figures are for a temperature of 37 to 38°C.

In addition to these factors there is also the question of the presence of pseudohemoglobin in the blood. According to Barkan (5) normal blood contains small amounts of two pigments, pseudohemoglobin and pseudomethemoglobin, which are intermediate in composition between ordinary hemoglobin and bile pigments. These pigments are distinguished and estimated by the ease with which metallic iron can be split from them in acid solution. Barkan deduces, by an indirect method, that the value of M for pseudohemoglobin is around 10 times greater than that for normal hemoglobin.

If the sum of the pO_2 and pCO is not sufficient to saturate the hemoglobin and there is an appreciable amount of reduced hemoglobin present, the amounts of COHb and O_2Hb at equilibrium can be worked out from the O_2-dissociation curve in absence of CO if Haldane's two basal assumptions are made:

A. That the amount of reduced hemoglobin present in a mixture of O_2 at partial pressure pO_2 and of CO at partial pressure pCO, is the same as it would be in absence of CO if the partial pressure of O_2 was equal to $pO_2 + MpCO$, M being defined as in equation 1. The amount of reduced hemoglobin can thus be read off from the O_2-dissociation curve in absence of CO.

B. That the hemoglobin combined with gas is partitioned between COHb and O_2Hb according to equation 1 even when there is appreciable reduced hemoglobin present.

The method of calculation now to be described embodies features drawn from previous treatments especially that of J. B. S. Haldane but is simpler and involves fewer assumptions than any of them. It may be most readily explained by working through a typical example.

Let figure 1, curve A, represent the dissociation curve of blood in absence of CO but at $pCO_2 = 40$ mm., pH = 7.4, 37°C.

It is required to find the pO_2 in equilibrium with the blood when [COHb] = 20 per cent, [O_2Hb] = 32 per cent, [Reduced Hb] = 48 per cent of the total hemoglobin.

We have [COHb] + [O_2Hb] = 20 + 32 = 52 per cent of the total hemoglobin.

From figure 1A it is seen that the gas pressure corresponding to 52 per cent = 27.4 mm.

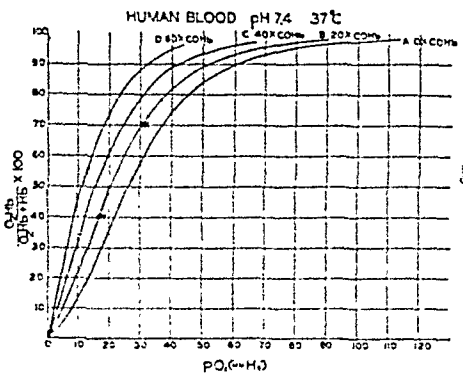

Fig. 1

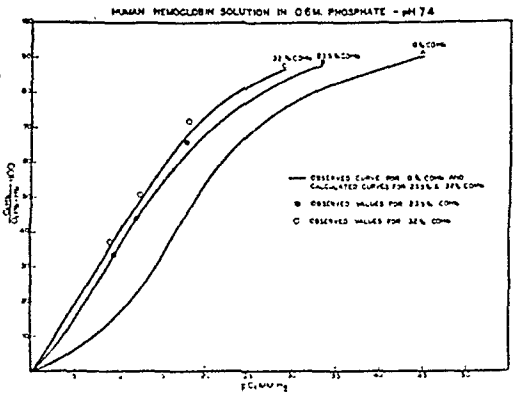

Fig. 2

Fig. 1. Oxyhemoglobin dissociation curves of human blood containing varying amounts of carboxyhemoglobin, calculated from the observed O_2-dissociation curve of CO free blood.

Fig. 2. Observed and calculated oxyhemoglobin dissociation curves of buffered human hemoglobin solutions containing varying amounts of carboxyhemoglobin.

Therefore:

$$pO_2 + MpCO = 27.4 \text{ mm.} = pO_2\left(1 + \frac{MpCO}{pO_2}\right) \tag{2a}$$

Now by equation (1):

$$\frac{MpCO}{pO_2} = \frac{[COHb]}{[O_2Hb]} = \frac{20}{32} \tag{2b}$$

Therefore:

$$pO_2 + MpCO = pO_2\left(1 + \frac{[COHb]}{[O_2Hb]}\right) = pO_2\left(1 + \frac{20}{32}\right) = 27.4 \tag{2c}$$

So $pO_2 = 16.9$ mm.

It will be seen that the calculated value of pO_2 is entirely independent of the values of M and pCO, which are in fact irrelevant. This result is made use of in the experimental section and is further considered in the discussion.

Since

100 × per cent O_2Hb/(per cent O_2Hb + per cent Red. Hb) =
100 × 32/(32 + 48) = 40 per cent

by plotting 40 against 16.9, we obtain a point on the O_2-dissociation curve of the remaining 80 per cent of the blood hemoglobin which is not combined with CO, i.e., *Y* of figure 1B.

Similarly for [COHb] = 20 per cent, [O_2Hb] = 56 per cent, [Red. Hb] = 24 per cent of the total hemoglobin we have

$$pO_2 = 41.2\text{mm.}/(1 + \tfrac{20}{56}) = 30.4 \text{ mm. and } 100 \times \text{per cent } O_2\text{Hb}/(\text{per cent } O_2\text{Hb} + \text{per cent Red. Hb}) = 100 \times 56/(56 + 24) = 70 \text{ per cent}$$

thus obtaining a second point on the O_2 dissociation curve at constant COHb concentration = 20 per cent, i.e., *X* on figure 1B.

Repetition of this procedure for other selected values of the per cent O_2Hb enables the whole O_2-dissociation curve at 20 per cent COHb to be plotted as in figure 1, curve *B*.

Exactly the same method of calculation can then be applied for COHb percentages, e.g., of 40 per cent, 60 per cent (fig. 1, curves *C* and *D*) or of any other desired value. From the empiric O_2-dissociation curve in absence of CO we thus readily derive a family of curves relating pO_2 to [O_2Hb]/([O_2Hb] + [Red. Hb]) at selected constant values of [COHb], without making any assumption at all as to the equation of the O_2-dissociation curve.

Our method therefore seems preferable to most previous treatments since in these the validity of Hill's equation for the dissociation curve is assumed. This equation now has no theoretical basis and can only be used as an empirical expression over the middle of the curve, but not at the extremes.

EXPERIMENTAL. A. *Hemoglobin solutions.* The following data were taken from our earlier work (9) on hemoglobin solution in which the shifts in the O_2-dissociation curves due to COHb and methemoglobin were compared, but the shift due to COHb was not analyzed at that time in relation to the Haldane theory.

Cleared human hemoglobin solutions in 0.6 M phosphate solution, pH 7.4 at 37°C. were used. The mixtures of COHb and O_2Hb were made by first equilibrating a portion of the solution with CO (tension = approx. 50 mm. Hg) and then mixing this solution with O_2Hb in the desired proportion. The mixture was then divided into several portions and rotated with various mixtures of O_2 and N_2. After this second equilibration of one or two hours, the liquid phases were analyzed for O_2 and CO and the gas phases for O_2. It was found that negligible CO had been lost from the liquid phase during this equilibration. It is certain that one to two hours' rotation were enough to bring the O_2 in the gas phase and the liquid phase into equilibrium with one another and with the hemoglobin. It is also most probable that equilibrium was complete between the hemoglobin and the dissolved CO, since at 37°C. Roughton's (20) observations show that the half-time of chemical dissociation of COHb is only a few seconds. On the other hand, owing to the low pressure gradient of CO between the blood and gas phases it seems equally clear (from calculations like those of Roughton (21) on the rate of gas-liquid exchange in manometric reactions) that it would take many hours for diffusion to bring the CO in the gas phase to its final equi-

librium value. This latter lack of equilibrium would, however, only matter if the theory required an exact knowledge of the equilibrium pCO of the gas phase; this, as pointed out above, is not the case—all the theory requires is that the equilibrium *in the blood* between the Hb, O_2 and CO should be complete and that the per cent O_2Hb, per cent COHb, and equilibrium pO_2 should be known. These factors are given by the blood-gas analyses. Technically it is a great advantage not to have to continue the tonometer equilibration for many hours, since possibility of decomposition of the hemoglobin, and, in the case of whole blood, of pronounced glycolysis, is thereby reduced.

Figure 2 presents a summary of these data. The three curves reading from right to left are, *A*, the measured dissociation curve of O_2Hb in the absence of CO; *B*, the curve calculated according to theory for dissociation of O_2Hb in the presence of 23.5 per cent COHb, and *C*, the same type of calculated curve in the presence of 32 per cent COHb. The solid circles (●) represent actual experimental points of a solution containing 23.5 per cent COHb. The test of the theory is the closeness with which these points approach curve *B*. Similarly the open circles (o) represent experiments on a solution containing 32 per cent COHb and should lie on curve *C*, if the theory is correct.

It will be seen that four of the six experimental points lie on the theoretical curve well within the experimental error. The other two points do not exactly fit the theoretical curve but are not sufficiently distant to rule out some technical error, especially since these experiments were not checked at the time to test this point.

B. *Human blood.* Blood of a single subject was used throughout in four experiments. It was drawn in the morning from an antecubital vein, mixed with heparin and placed in the icebox, from which portions were removed when needed. Fresh blood was drawn for each day's experiments.

A careful O_2 dissociation curve of each sample of drawn blood was determined with special attention to the upper half of the curve (from which the curves for partial conversion to COHb are chiefly calculated). In the first three experiments the tonometers for equilibration were made up with a pCO_2 of 40 mm. Hg, in the fourth with $pCO_2 = 25$ (in addition to the desired pO_2).

Although the subject did not smoke for 12 hours prior to bleeding, there was always found a small per cent of COHb (4 to 5.5 per cent) in his blood. On figure 3 the curves of his blood are corrected to 0 per cent COHb according to the theory in the introduction, and from these curves the theoretical curves are calculated for the desired per cent COHb. It may be noted that even at the same pH there is slight day to day variation in the position of the curve; thus it was important to determine the curve at 0 per cent COHb for each day's experiment.

The experimental equilibrium points at the chosen per cent of COHb were determined in a manner similar to that used for hemoglobin solutions. A portion of the blood was equilibrated with N_2 containing CO to a pressure of 50 mm. Appropriate mixtures of this CO blood were made in a syringe with some of the original blood and stored in the ice-box for several hours. This allows time for

diffusion to bring about a uniform distribution of the CO between the red cells, which were initially saturated with the gas, and those which at the start were practically CO-free. The mixture was then equilibrated in a series of tonometers made up with varying O_2 pressures and a pCO_2 equal to that used on the 0 per cent COHb curve of that day. The equilibrium time at 37° was 30 minutes. As with the hemoglobin solutions the CO of the blood was found not to diffuse into the gas phase to an appreciable extent. On theoretical grounds one should again expect equilibrium within the liquid phase but not between liquid and gas in this time. For purposes of testing the theory the latter as shown above is not necessary.

Experiments 1, 2 and 3 were done with a pCO_2 of 40, thus giving a pH of somewhat lower than 7.4 and approximating blood conditions in vivo at sea level. For experiment 4 a pCO_2 of 25 was chosen to approximate the extreme conditions in vivo with hyperpnea such as would occur at high altitudes.

All blood samples were analyzed for CO_2, O_2 and CO by the methods of Van Slyke-Neill with some modifications of Horvath and Roughton (16). Tonometer gases were analyzed for O_2 and CO_2. From these data serum CO_2 and pH_s were calculated for each blood sample using the line chart of Peters and Van Slyke and the Henderson-Hasselbalch equation. The mean pH of the CO-containing bloods was taken as standard for the experiment. Among these CO-containing bloods the pH_s never varied more than 0.02 from the mean, but the points on the curve of the blood as drawn were usually slightly less acid (due to shorter equilibration and therefore less glycolysis). The curve of 0 per cent COHb usually needed correction to reach the chosen standard pH_s; for this the empirical relationship of Dill et al. (10) was used (Δ log. $pO_2 = -0.48\Delta$ pH). For example, the observed value for one of the points with 0 per cent COHb in experiment 3 was 60.5 per cent O_2Hb at $pO_2 = 34.4$ and $pH_s = 7.42$. For correction to $pH_s = 7.36$, $\Delta\ pH_s = -0.06$. Therefore, $\Delta \log pO_2 = -0.48 (-0.06) = +0.029$, and corrected pO_2 = antilog (log 34.4 + 0.029) = 36.0.

Figure 3 presents the results of the four experiments, presented similarly to figure 2. The solid lines on the right in each graph present the measured O_2 dissociation curve of the CO free blood. The one or two solid lines on the left are the theoretical curves calculated from the right hand curve. The points plotted are the actually observed values of the CO-containing bloods. As before the test of the theory is the closeness of these points to the corresponding theoretical curve.

As with the hemoglobin solutions, it will be seen that the great majority of the points lie close to or on the theoretical curves. Only two points out of a total of 26 (both in expt. 2) are clearly distant from the curves. We have no explanation for these discrepancies, but attribute them to undetected errors in our procedures, since the agreement is generally good in experiment 3, which is a repetition of experiment 2. Dissociation curve experimentation of this kind is in fact a somewhat exacting task, especially when done in hot and humid weather as were these experiments; in previous work it has not been uncommon for one or two points out of ten to be distinctly out of line with the remainder, due presumably to human fallibility.

DISCUSSION. *Physico-chemical.* The treatment based on the assumptions A and B (v. review of Haldane theory on p. 18) was shown above to lead to deduction of the effect of a given per cent COHb without any assumption as to the numerical value either of M or of pCO at equilibrium. Independence of M indicates that the COHb effect would not be changed either by any of the factors on which M depends, namely, temperature, illumination, species, or even in mixtures of two pigments of different M values, as may occur in blood when appreciable amounts of pseudohemoglobin (Barkan) are present. The independence of pCO has already proved very advantageous in the experimental procedure. The whole treatment is thus more simple and comprehensive than

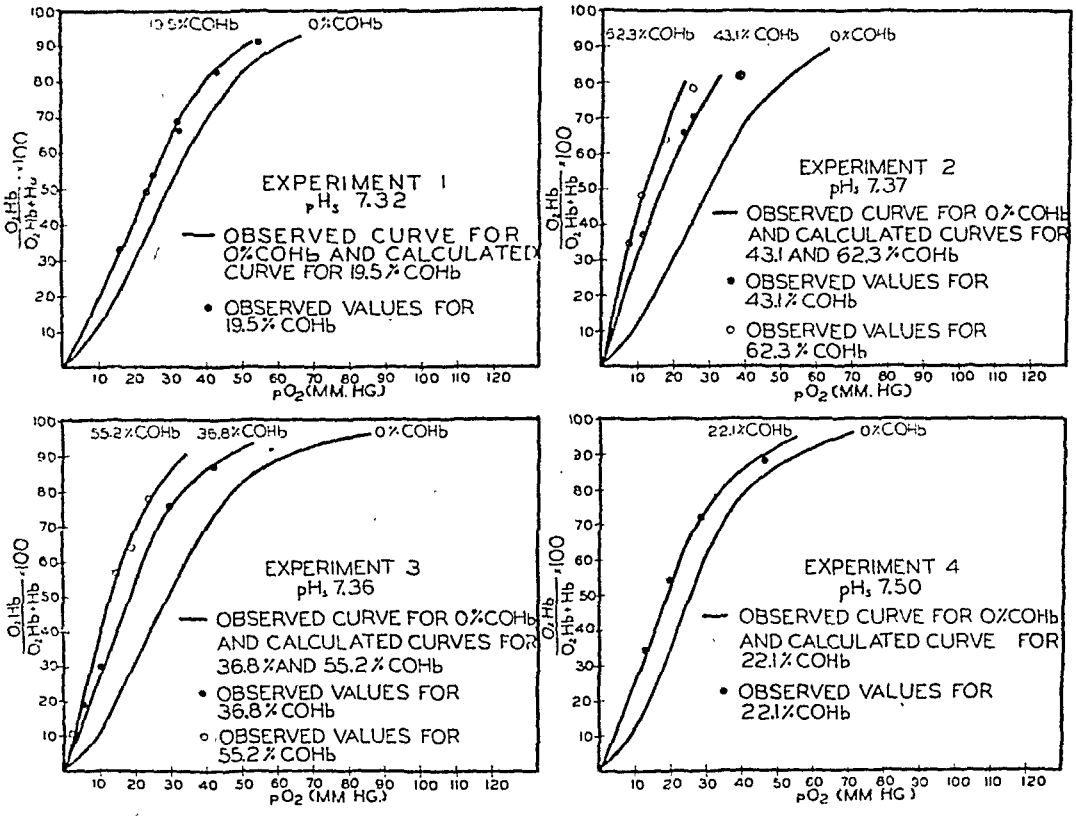

Fig. 3. Observed and calculated oxyhemoglobin dissociation curves of human blood containing varying amounts of carboxyhemoglobin.

those based on pCO and numerical value of M (e.g., Stadie and Martin, Van Slyke, Barcroft); the only disadvantage is that it does not inform us of the values of pCO at equilibrium. These, however, are not necessary for testing the theory nor are they of much physiological importance since it is rare for a subject to go on breathing a CO-containing atmosphere long enough to reach complete equilibrium.

So important are assumptions A and B for the whole problem that it is necessary to inquire how they stand in regard to the physico-chemical theories of the O_2-hemoglobin equilibrium. Their relation to two of the older theories has already been considered by Haldane and A. V. Hill. The subsequent measurements of the osmotic pressure and molecular weight of hemoglobin by Adair

(1, 2) and by Svedberg (24) have, however, shown that the main postulates both of the Haldane and the Hill theory are invalid; the only offshoot of either of these theories which still survives in the literature is Hill's equation for the O_2-hemoglobin equilibrium, this being retained and used only on account of its convenience as an empirical expression. On the other hand, wide acceptance has been and is still given to the intermediate compound hypothesis, first put forward in general form by Adair (1, 2,) and subsequently developed along a special and very fertile line by Pauling (18). According to Adair's hypothesis the reaction between O_2 and hemoglobin takes place in four stages:

$$O_2 + Hb_4 \rightleftharpoons Hb_4O_2,\ [Hb_4O_2] = K_1\,[O_2]\,[Hb_4] = K_1pz \tag{3}$$
$$O_2 + Hb_4O_2 \rightleftharpoons Hb_4O_4,\ [Hb_4O_4] = K_2\,[O_2]\,[Hb_4O_2] = K_1K_2p^2z \tag{4}$$
$$O_2 + Hb_4O_4 \rightleftharpoons Hb_4O_6,\ [Hb_4O_6] = K_3\,[O_2]\,[Hb_4O_4] = K_1K_2K_3p^3z \tag{5}$$
$$O_2 + Hb_4O_6 \rightleftharpoons Hb_4O_8,\ [Hb_4O_8] = K_4\,[O_2]\,[Hb_4O_6] = K_1K_2K_3K_4p^4z \tag{6}$$

where $[O_2] = p$, $[Hb_4] = z$ and K_1, K_2, K_3, K_4 are the equilibrium constants of the respective reactions. Similarly the reaction of CO with hemoglobin takes place in four stages: $CO + Hb_4 \rightleftharpoons Hb_4CO$ (equilibrium constant L_1), $CO + Hb_4CO \rightleftharpoons Hb_4(CO)_2$ (equilibrium constant L_2), etc. The fact that the dissociation curve of oxyhemoglobin agrees exactly with that of carboxyhemoglobin if the scale of gas pressures is altered M-fold is most simply explained by assuming $L_1/K_1 = L_2/K_2 = L_3/K_3 = L_4/K_4 = M$. Roughton (20) has applied the intermediate compound hypothesis to the equilibrium between CO, O_2 and Hb when the pressures of the two gases are such as to saturate the hemoglobin almost completely: no previous attempt has been made, so far as we know, to handle, on the basis of the intermediate compound hypothesis, the equilibrium between CO, O_2 and Hb when appreciable amounts of reduced hemoglobin are present, as in the experimental work of this paper. We shall now show that assumptions A and B fit in readily with the intermediate compound hypothesis, if a few additional and reasonable assumptions be made.

Consider first the intermediate compounds in which only one of the four gas-combining spaces of the hemoglobin molecules is occupied, Hb_4O_2 and $Hb_4(CO)$.

Let $[Hb_4] = z$, $[O_2] = p$, $[CO] = q$

Then $[Hb_4O_2] = K_1pz$ and $[Hb_4(CO)] = L_1qz$

From this it follows that the ratio of combined CO to combined O_2 = $[Hb_4(CO)]/Hb_4O_2] = L_1q/K_1p = \frac{Mq}{p}$, i.e., assumption B holds.

Also the total number of molecules of combined gas $(O_2 + CO) = [Hb_4O_2] + [Hb_4(CO)] = (K_1p + L_1q)z = (K_1p + K_1qL_1/K_1)z = K_1\,(p + Mq)z$, which according to equation (3) is the same as the number of molecules of O_2 which would be so combined, if no CO was present but the O_2 pressure was equal to $(p + Mq)$, i.e., assumption A holds.

Next consider the intermediates containing 2 molecules of gas, i.e., Hb_4O_4, $Hb_4O_2\,(CO)$, $Hb_4(CO)_2$.

We then have $Hb_4O_4 = K_1K_2p^2z$

$$Hb_4(CO)_2 = L_1L_2q^2z$$

$$Hb_4O_2(CO) = K_1L'_2pqz$$

where L'_2 is the equilibrium constant of the reaction $CO + Hb_4O_2 \rightleftharpoons Hb_4O_2(CO)$

$$= \frac{\text{velocity constant of reaction } Hb_4O_2 + CO \rightarrow Hb_4O_2(CO)}{\text{velocity constant of reaction } Hb_4O_2(CO) \rightarrow CO + Hb_4O_2}$$

We now introduce the new assumptions to which we have already referred. Let us assume that the velocity constant of the reaction $CO + Hb_4O_2 \rightarrow Hb_4O_2(CO)$ is the same as that of the reaction $CO + Hb_4CO \rightarrow Hb_4(CO)_2$, i.e., the chance of CO combining with a molecule of hemoglobin in which one of the four spaces is already occupied is the same whether that one space is occupied by O_2 or CO: furthermore, assume on the same general grounds that the velocity constant for the dissociation reaction $Hb_4O_2(CO) \rightarrow Hb_4O_2 + CO$ is half that of the dissociation reaction $Hb_4(CO)_2 \rightarrow Hb_4CO + CO$ since in the latter case there are two CO molecules available to dissociate whereas in the former there is only one.

If these assumptions are accepted $L'_2 = 2L_2$

The ratio of combined CO radicals to combined O_2 radicals in the intermediates containing two molecules of gas then

$$= \frac{[Hb_4O_2(CO)] + 2[Hb_4(CO)_2]}{2[Hb_4(O_2)_2] + [Hb_4O_2(CO)]} = \frac{K_1L'_2pqz + 2L_1L_2q^2z}{2K_1K_2p^2z + K_1L'_2pqz}$$

$$= \frac{2K_1L_2pqz + 2L_1L_2q^2z}{2K_1K_2p^2z + 2K_1L_2pqz} = \frac{K_1L_2q(p + L_1q/K_1)}{K_1K_2p(p + L_2q/K_2)}$$

$$= \frac{K_1L_2q(p + Mq)}{K_1K_2p(p + Mq)} = \frac{Mq}{p}$$

i.e., assumption B holds for the 2-molecule containing intermediates as well as for the 1-molecule containing intermediates.

The total number of gas molecules combined in the 2-molecule intermediate form

$$= 2[Hb_4O_4] + 2[Hb_4O_2(CO)] + 2[Hb_4(CO)_2] = 2K_1K_2p^2z + 2K_1L'_2pqz + 2L_1L_2q^2z$$

$$= 2K_1K_2p^2z + 4K_1L_2pqz + 2L_1L_2q^2z = 2K_1K_2z(p^2 + 2pqL_2/K_2 + q^2L_1L_2/K_1K_2)$$

$$= 2K_1K_2z\,(p^2 + 2Mpq + M^2q^2) = 2K_1K_2\,(p + Mq)^2z$$

Thus the total number of gas molecules combined both in the 1-molecule and the 2-molecule containing intermediate $= K_1(p + Mq)z + 2K_1K_2(p + Mq)^2z$. This according to equations (3) and (4) is the same as the number of molecules of O_2 which could be so combined if no CO was present and the O_2 pressure was equal to $(p + Mq)$, i.e., assumption A again holds.

The same arguments can in turn be applied to the 3-molecule and 4-molecule containing intermediates: the calculation process becomes progressively more complicated but the end result is the same, namely, the maintenance of assumptions A and B. Thus with relatively few and reasonable auxiliary assumptions the two main postulates on which the calculation of the O_2-hemoglobin dissociation curve in presence of CO is based, can be reconciled with the general form of the intermediate compound hypothesis proposed by Adair. The same result is reached with Pauling's special form of the intermediate compound hypothesis if similar reasoning and auxiliary assumptions are used, so the detailed working out in this case may be left to the reader who wishes to undertake it. It may be noted that Pauling's interaction constant, α, must be assumed to remain the same whether the neighboring molecule which exerts its interaction effect is O_2 or CO.

In a recent paper we have shown that partial conversion to methemoglobin also shifts the O_2-dissociation curve of the remaining curve to the left. In five out of eleven cases the shift was almost exactly equal to that found with the same amount of COHb, but in the other six cases the shift was only about half as great. The causes of this variability have not yet been worked out, but at all events we feel convinced that the explanation of the methemoglobin effect must be qualitatively of the same type as that of the COHb effect. Further support for this is given by our observation that the two effects were additive. Conant and Fieser (6) suggested that the equilibrium between O_2, O_2Hb, MetHb, ferricyanide and ferrocyanide could be expressed by the equation:

$$\frac{[\text{MetHb}]}{[\text{O}_2\text{Hb}]} = \frac{\text{N}[\text{ferricyanide}]}{\text{pO}_2[\text{ferrocyanide}]} \tag{7}$$

Equation (7) is analogous to equation (1), where N is an equilibrium constant and if similar assumptions to (A) and (B) hold good in the case of MetHb, the effect of the latter on the O_2-dissociation curve should be calculable by equations (2a), (2b) and (2c) with MpCO replaced by N [ferricyanide]/[ferrocyanide] and [COHb]/[O_2Hb] by [MetHb]/[O_2Hb]. The actual shift of the dissociation curve would thus only depend on the fraction [MetHb]/[O_2Hb] and not on N, [ferricyanide] and [ferrocyanide] except insofar as these determine [MetHb]/[O_2Hb]. It should furthermore be the same for a given [MetHb] as for the same [COHb], as indeed we found in 5 out of 11 cases.

The actual values of N, as calculated by Conant and Fieser from a series of experiments in which Hb was treated with various amounts of ferricyanide and equilibrated with O_2, show, however, a rather wide scatter. In subsequent data by Conant and Scott (7) on the corresponding equilibrium between CO, COHb, MetHb, ferricyanide and ferrocyanide, no actual calculations of the equilibrium constant are quoted by the authors. The figures given by them are, however, adequate for this purpose and we have worked out the value of the equilibrium constant = pCO[MetHb] [ferrocyanide]/[COHb] [ferricyanide] from their experimental data on solutions equilibrated with pure CO. The values of the constant unfortunately show a scatter of at least 10-fold. It therefore seems scarcely

safe at present to apply any quantitative theory on this basis to the effect of MetHb on the O_2-dissociation curve.

PHYSIOLOGICAL DISCUSSION. In subjects exposed to carbon monoxide, two important problems arise. 1. How fast does the per cent COHb in the blood increase? 2. What is the effect of a given per cent COHb in the blood on the loading and unloading of O_2? The rate and extent of the rise of per cent COHb depends upon the pCO, pO_2 in the air breathed, the time of exposure and the ventilation rate. These factors have already been investigated in other laboratories and are to be dealt with more fully in forthcoming papers from this laboratory. We shall here concern ourselves with problem 2.

To begin with we must establish that the effect of COHb on the O_2-dissociation curve as determined in vitro in the experimental section also applies quantitatively in circulating blood during gas exchange in the capillaries. When there is no COHb in the blood, the O_2-dissociation curve of the blood as determined in vitro is generally assumed to apply also in vivo, since the rate of exchange of O_2 between chemical combination with Hb and physical solution in the red cell and plasma is known to be so fast. The presence of appreciable COHb introduces two possible complications: 1, that its amount might change during the passage of the blood through the capillary, and 2, that the distribution of CO between chemical combination and physical solution might not be fast enough for the equilibrium state to be preserved throughout the capillary phase. In regard to the first point the diffusion pressure of dissolved CO in the blood is so low that it is unlikely that any gain or loss in total CO of the blood could occur during a single passage of blood through the tissue capillary except perhaps in the case of muscle, where there is possibility of very rapid combination with the appreciable amounts of myoglobin contained therein. That the average change for all the capillaries of the body is not significant is shown by the observation that in the whole animal the per cent COHb in the blood after exposure to CO normally takes several hours to drop to half its value. As regards the second point it is true that the unloading of O_2 in the capillary leads to an increased concentration of reduced hemoglobin in the blood which in turn will cause transfer of a minute amount of CO from physical solution to combination with Hb. Suppose in a typical instance that blood entering the capillary contains 30 per cent COHb, 67 per cent O_2Hb, $pO_2 = 100$ mm. Hg, $pCO = 0.21$ mm. Hg, and leaves the capillary with 35 per cent O_2Hb. Reference to figure 1 and to equations (2a), (2b) and (2c) shows that the pO_2 in the venous blood will be 19 mm. and the $pCO = 0.08$ mm. The drop in pCO will thus only amount to 0.13 mm. and the corresponding rise in per cent COHb will be only about 0.002. From Roughton's data it can be calculated that the time for such a change is about 0.01 sec., which is 1/100 or less than the average time spent by the blood in the capillary. At each instant during passage of blood through the capillary the CO as well as the O_2 is therefore effectively in equilibrium between chemical combination and physical solution. As regards O_2 distribution the condition of the blood accordingly traverses the O_2-dissociation corresponding to the assigned [COHb] of the blood. As regards CO, the [COHb] remains effectively constant whereas the

pCO traverses a course which cannot be measured directly but can be calculated as in the example just given. All these considerations make it evident that the evaluation of the shift of the O_2-dissociation curve in terms of the COHb content is much more apposite from the physiological viewpoint as well as more convenient from the experimental angle than is its evaluation in terms of pCO.

The smaller amount of hemoglobin available for O_2 transport in CO poisoning together with the shift of the dissociation curve of the residual Hb to the left

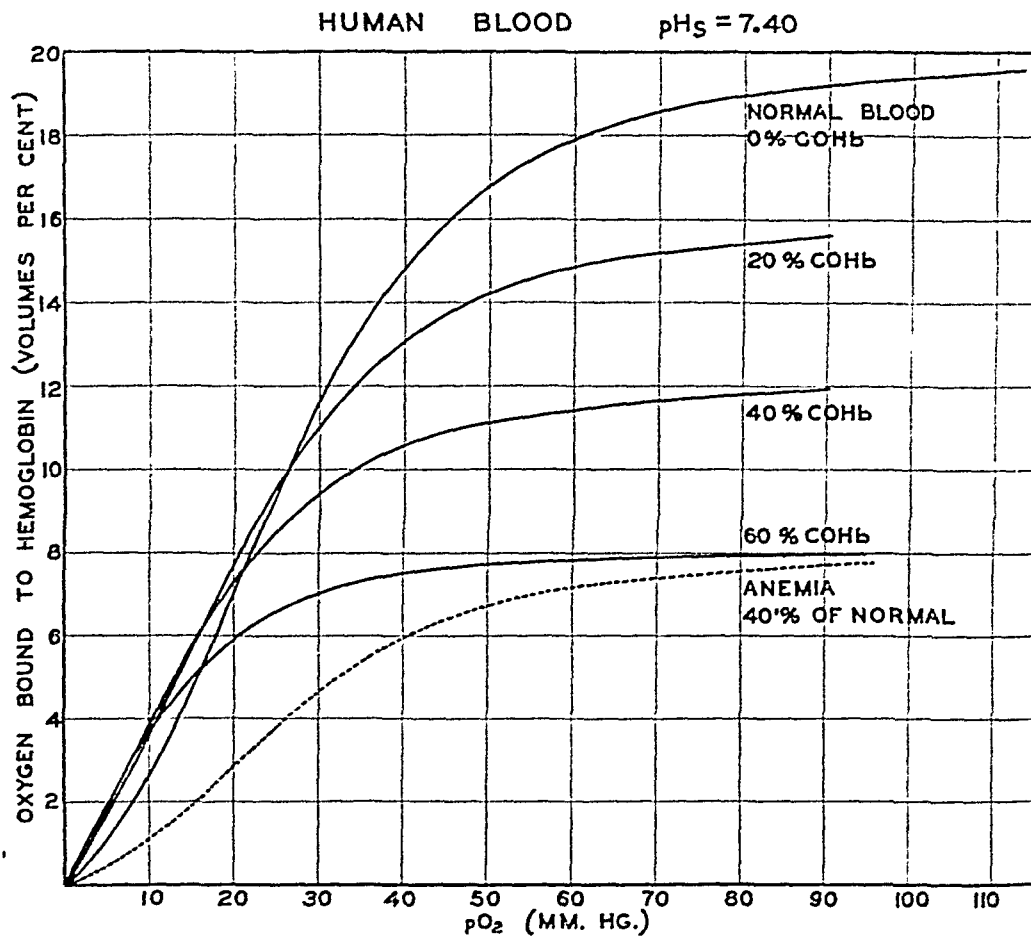

Fig. 4. Calculated O_2-dissociation curves of human blood containing varying amounts of carboxyhemoglobin, plotting the absolute amounts of bound O_2 rather than the percentage of available hemoglobin bound to O_2.

(cf. fig. 1) were considered by Haldane as additive factors in explaining the special handicap to the organism in O_2 unloading. Actually, however, the recent observations of Asmussen and Chiodi (3) do not indicate as severe a handicap as might be expected from this line of approach. Thus at rest, at light work and at heavy work 33 per cent COHb in the blood led to no appreciable change in the O_2 consumption per minute nor was any change found in the cardiac output, increase in which might have compensated for the adverse effect of change in the dissociation curve. In a later paper (8) such compensation was, however, shown to appear

in resting subjects when the per cent COHb exceeded 40. For these reasons we feel that the alternative method of plotting given in figure 4 (and independently used by Dr. J. R. Pappenheimer) is more appropriate for physiological use than that adopted by Haldane. Here the total amount of bound O_2 is plotted against pO_2 instead of the amount relative to the available hemoglobin as in figure 1. It will be seen that the curves for the various percentages of COHb are widely different at the higher O_2 pressures but are almost coincident at the lower pressures.

In normal man at rest at sea level only the upper half of the steep part of the O_2-dissociation curve is made use of in O_2 unloading, the lower half (i.e., 10–25 mm. pO_2) of the steep part being kept as a reserve, which is only called upon in exercise or in pathological conditions. In the presence of COHb it seems clear that this reserve could be called upon if the shape and portion of the O_2-dissociation curve as plotted in figure 4 in the 10 to 25 mm. pO_2 range was about the same as in the absence of COHb. Figure 4 shows that the dissociation curves over this low range are very much the same for all per cent COHb within 0 to 40, and therefore the O_2 uptake can be kept steady just by using up more or even all of the reserve normally available from the shape of the dissociation curve. Above 40 per cent COHb figure 4 shows that the type of reserve given by the dissociation curve rapidly becomes exhausted and it is therefore not surprising that a dangerous situation rapidly develops. The carotid sinus is generally supposed to be sensitive to the pO_2 rather than to the O_2 content of the arterial blood, and since in CO poisoning at sea level there is no change in arterial pO_2, there is no stimulus of the sinus to provide compensatory increase in heart output or ventilation, as there is when breathing air of low pO_2. The lack of such compensation, together with depletion of reserve, explains the sudden collapse at rest if the per cent COHb is increased above the critical level of 40 to 50 per cent COHb. Resting subjects at or even below this critical level frequently collapse if they take mild exercise: the reason for this is that the reserve, though adequate in rest, is no longer big enough to supply the increased need of the body in work.

The curve for 40 per cent anemia is also presented in figure 4 to demonstrate again the marked difference as regards O_2 unloading from that which obtains when 60 per cent of the Hb is combined with CO rather than merely absent. The anemic subject has the further advantage of lowered blood viscosity, which may lead in turn to an earlier and more pronounced compensatory increase in circulation rate than that found in CO-poisoning.

Figure 4 is also specially useful in another connection. At very low O_2 pressures Haldane and L. Smith (12) made the startling observation that mice might be actually benefited by the addition of small amounts of CO in the inspired air. Haldane explained this in terms of the appreciable increase in O_2 which the blood would take up when exposed to a low loading O_2 tension in the presence of COHb, owing to the shift to the left in the dissociation curve produced by the latter. We must also, however, consider how much O_2 remains bound to the Hb at the unloading tension at the venous end of the capillary, since no benefit will result to the organism unless the difference between the bound O_2 at the beginning and

end of the capillary is increased. Thus with an arterial pO_2 of 20 mm. and a venous pO_2 of 12 mm., the O_2 unloaded is, (v. fig. 4) 3.5 vols. per cent in the case of CO-free blood, is only 3.2 vols. per cent in the case of blood containing 20 per cent COHb. On the other hand the O_2 unloaded between an arterial pO_2 of 12 mm. and a venous pressure of 4 mm. is 2.7 vols per cent for CO-free blood and 2.9 vols. per cent in the case of 20 per cent CO-blood. At pressures above 20 mm. the unloading from CO-free blood is progressively better than from CO-containing blood; it is only in the region of the lower inflection of the dissociation curve that the opposite effect is seen to occur. Since pressures in the neighborhood of 12 mm. are lower than man can tolerate, it does not appear possible that the Haldane-Smith effect could ever be observed in man. In the case of mice, however, the O_2-dissociation curve of the blood is shifted greatly to the right of man, the affinity being only about one-third in this region: the critical O_2 tension at which a beneficial effect of CO might occur would therefore be expected to be about 3 times greater, namely, at 36 mm. Haldane's observations have shown that mice in absence of CO can withstand O_2 pressures in the inspired air as low as 36 mm., and so in this species the Haldane-Smith effect should manifest itself quite definitely. From this method of attack it will be seen that whether or not any given species of animal will benefit from small amounts of CO when exposed to very low O_2 pressures will depend both on the position of the O_2-dissociation curve and on the critical value of O_2 pressure in the inspired air at which brain function fails, i.e., the oxygen ceiling.

CONCLUSIONS

1. A simplified method is proposed for calculating the O_2-dissociation curve of O_2Hb in the presence of a given per cent of COHb. It utilizes the observed dissociation curve of O_2Hb without CO and the theoretical assumptions of the partition of Hb between O_2 and CO; but avoids any equation for the hemoglobin dissociation curve and does not require the choice of a numerical value for the partition coefficient M.

2. The theory was tested in hemoglobin solutions in 0.6 M phosphate buffer pH 7.4 at two percentages of COHb and found to agree excellently with four out of six observed points, and the discrepancy in the remaining two was not very serious.

3. Whole blood showed generally excellent agreement between theory and observation when similarly tested at $pCO_2 = 40$ per cent and COHb approximately 20, 40, 60 per cent, and at $pCO_2 = 25$; per cent COHb = 22.

4. The relation of the fundamental assumptions of the theory to the intermediate compound hypothesis of the O_2-hemoglobin equilibrium is worked out.

5. It is shown that the effect of COHb on the O_2-dissociation curve in vivo should be quantitatively the same as the experimentally observed effect in vitro.

6. Plotting of O_2 pressure against total bound O_2 rather than against the fraction of the available hemoglobin bound with O_2 is shown to give clearer indications of the effect of COHb on the transport of O_2. In particular the conditions

under which *a*, O_2 supply would begin to fail, and *b*, small amounts of CO would have a beneficial effect at very low O_2 pressures (Haldane-Smith effect) are demonstrated.

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COMPARISON OF CARDIAC AND METABOLIC ACTIONS OF THYROXINE, THYROXINE DERIVATIVES[1] AND DINITROPHENOL IN THYROIDECTOMIZED RATS

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Received for publication October 30, 1943

It is generally believed that the various functions of the thyroid gland, including its effect on the heart, are the result of the metabolic stimulation produced by the thyroid secretions (1), and that thyroxine, as such or as prosthetic group in a complex substance, is the hormone responsible for all these effects (2–5). Recently, however, Meyer and co-workers have claimed that thyroxine does not have a stimulating effect on the heart proportional to its ability to augment metabolism; the effective cardiotropic agent of the thyroid was considered to be present in an acid-soluble fraction of the gland (6–10).

Because of the possibility of error in determination of the cardiac rate in rats by the method of palpation employed by Meyer, it was decided to estimate the heart rate by means of the electrocardiograph after administration of thyroxine or various other thyroid compounds. In addition, the effect of dinitrophenol was examined in order to evaluate the cardiac effect of another type of metabolic stimulant.

METHODS. Male rats weighing 130 to 150 grams were thyroidectomized and treated with various substances in the doses indicated in table 1, administered in two daily subcutaneous injections starting from the day of operation. The substances were dissolved in water with as little sodium hydroxide as possible, with the exception of diiodotyrosine, which was given in suspension in water or in N/100 NaOH. Synthetic *dl*-thyroxine was used in doses of 3 to 3000 micrograms daily. The greatest dose of thyroxine administered, 3000 micrograms daily, proved lethal in 5 of 7 animals. The experiment was carried out in June and July (room temperature in the vicinity of 28°C.) so that heat may have been a contributing factor in these deaths.

The heart rate was determined by the electrocardiographic method at 8, 15 and 21 days after starting treatment. Electrocardiograms were obtained by means of needle electrodes inserted under the skin on each side of the chest, and were recorded on a Sanborn Cardiette in some instances and in others by means of a General Electric Oscillograph and suitable amplifiers. Records were taken first while the animals were fully conscious and restrained manually or on a board, and later within 15 to 25 minutes after intraperitoneal injection of 30 mgm. of nembutal per kgm. The rectal temperature was taken in the anesthetized animals between the 5th and 15th minute after nembutal injection.

[1] We are grateful to Mr. Paul Blanc, Director, Hoffmann-La Roche, Limited, Montreal, for the thyroxine derivatives employed in these studies.

Finally the oxygen consumption was determined by the method of Williams, Phelps and Burch (11), and the rate of respiration was recorded. Although nembutal somewhat depresses the metabolic rate, regular and consistent results were obtained in our experimental conditions (28°C.).

Dinitrophenol was administered subcutaneously in doses of 15 mgm. per kgm. of body weight twice daily during the first two weeks after operation and three times daily during the third week. The measurements reported in table 1 were obtained before the first morning injection, and showed that the increased metabolism due to the drug had completely disappeared by that time. It was therefore necessary to perform acute experiments in which the heart rate was determined at the height of metabolic stimulation due to dinitrophenol. Eight animals (4 normal and 4 thyroidectomized rats) received 15 mgm. of dinitrophenol per kgm. by intraperitoneal injection (table 2); and four animals, including 2 normal and 2 thyroidectomized, received a single dose of 40 mgm. of dinitrophenol per kgm.; the latter dose was lethal in three of them (2 normal, 1 thyroidectomized). In all these animals the heart rate, breathing rate, metabolic rate, and body temperature were recorded at intervals of 15 minutes for 1 to 2 hours.

Results. Thyroidectomy produced a decrease in the heart rate whether the animals were restrained or anesthetized (table 1). A significant diminution was observed in body temperature and oxygen consumption but not in rate of respiration.

The cardiac rates of animals receiving thyronine, dinitrophenol, potassium iodide, and diiodotyrosine did not differ significantly from those found in the thyroidectomized group. Diiodothyronine (412 micrograms daily) raised the heart rate to the normal range. A daily dose of 3 micrograms of thyroxine also produced a significant increase in the heart rate. The values obtained were comparable to the lower ones encountered in normal controls. Body temperature and rate of respiration closely approached normal values. With 30, 300 and 3000 micrograms of thyroxine, supernormal values, increasing with the dose, were observed for body temperature, respiratory rate, metabolism, and heart rate. The values for heart rate in conscious restrained animals were consistently higher than after anesthesia. The magnitude of this difference varied considerably in the several groups, but these variations did not seem significant.

The values found in each group were similar at 1, 2 or 3 weeks after the beginning of injection (fig. 1). However, in thyroidectomized animals receiving no therapy or ineffective therapy the values for the heart rate kept on decreasing during the three weeks, although the decrease was smallest during the third week. The thyroxine-groups showed a low point at the second week, the significance of which is doubtful.

The lack of effect of dinitrophenol on the heart observed in the experiment summarized in table 1 was also found during the period of metabolic hyperactivity (table 2). In doses of 15 mgm. per kgm. the drug doubled or almost doubled metabolism and the rate of respiration, while variations in heart rate were of doubtful significance (table 2). A definite effect on the heart was

noted only after injection of a lethal dose of dinitrophenol (40 mgm. per kgm.). In these conditions the body temperature rose to over 40°C. in 2 normal rats and to 39 and 37.5°C. respectively in 2 thyroidectomized rats; the breathing rate was doubled or tripled in all. The maximal heart rate found after injection represented an increase of 14 and 65 per cent in the 2 normal, and of 29 and 0 per cent in the 2 thyroidectomized rats respectively. Therefore, a rise in metabolism, which was high enough to have caused 3 of the 4 animals to die,

TABLE 1

Effect of thyroxine and derivatives on body temperature, oxygen consumption, and heart rate, after a 21-day treatment

	Number of animals	Daily dose	Iodine content of drug given	Average body temperature	Average breathing rate	Oxygen consumption	Heart rate		
							Nembutal	Restrained	Difference
		mgm.	*mgm. per day*	*degrees C.*	*per min.*	*liters per sq.m. per day*	*per min.*	*per min.*	*per min.*
Control	10			37.5±0.1	64± 7	154	379± 9	431±21	+52
Thyroidectomy	9			36.7±0.2	60± 6	116	265±12	339±11	+74
Thyroidectomy and thyronine	6	0.825	0	36.5±0.1			289±28	320±25	+31
Thyroidectomy and dinitrophenol	7	4.2–6.3	0	36.5±0.2	52± 9	104	254± 7	367± 7	+123
Thyroidectomy and potassium iodide	5	7.670	5.850	36.7±0.2			303±20	394±18	+91
Thyroidectomy and diiodotyrosine	10	10.000	5.850	37.0±0.1	55± 8	115	293±11	370±22	+77
Thyroidectomy and diiodothyronine	7	0.412	0.200	37.1±0.3			371±28	411±13	+40
Thyroidectomy and 3γ thyroxine	6	0.003	0.002	37.2±0.1	72± 5	148	344±21		
Thyroidectomy and 30γ thyroxine	4	0.030	0.020	38.1±0.1	110±10	254	478±13	527±10	+49
Thyroidectomy and 300γ thyroxine	8	0.300	0.200	38.2±0.2	101± 8	321	505±17	558±18	+53
Thyroidectomy and 3000γ thyroxine	2	3.000	2.000	38.6			574	647	+73

increased the heart rate markedly in 1 animal, slightly in 2, and not at all in a fourth. Incidentally, it was noted here, as is also apparent in table 2, that the metabolic stimulation was less and of slower onset in the thyroidectomized rats than in intact controls.

Discussion. The heart rates found here are in agreement with the electrocardiographic values found in normal (12, 13, 14) and thyroid-treated (15, 16) rats, but not with the figures obtained by the methods of palpation (5, 6–10).

Thus Meyer and co-workers reported that the heart rate of thyroidectomized rats determined by palpation varied between 170 and 200 beats per minute; with daily doses of 3 micrograms of thyroxine per 10 grams of body weight for

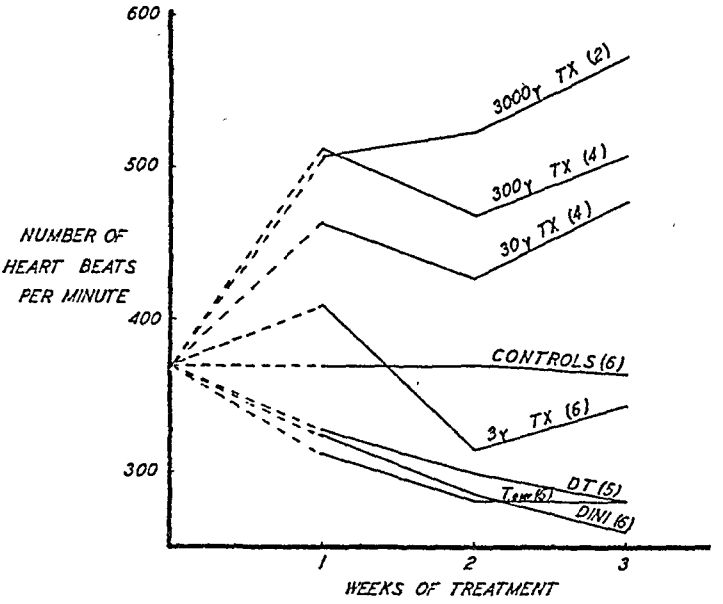

Fig. 1. Relation of heart rate in thyroidectomized rats (nembutal anesthesia) to dosage and duration of treatment with thyroxine, *TX*, diiodotyrosine, *DT*, dinitrophenol, *DINI;* as compared to intact animals (CONTROLS) and untreated thyroidectomized rats, *TOMY*. Each group was started with 6 animals. Figures in parentheses indicate the number of survivors, from which the figures used in the chart were obtained.

TABLE 2

Effect of injection of 15 mgm. of dinitrophenol per kgm. on heart rate, breathing rate, and oxygen consumption

	BEFORE INJECTION		30 MINUTES AFTER INJECTION			MAXIMAL VALUES FOUND		
	Heart rate	Breathing rate	Heart rate	Breathing rate	Oxygen consumption increase (over preinjection value)	Heart rate	Breathing rate	Oxygen consumption increase (over preinjection value)
	per min.	*per min.*	*per min.*	*per min.*	*per cent*	*per min.*	*per min.*	*per cent*
Normal	341 (278–412)	62 (44–79)	356 (326–482)	113 (90–136)	86 (80–92)	379 (312–450)	123 (95–136)	118 (113–122)
Thyroidectomized	245 (227–262)	53 (38–78)	245 (214–263)	84 (66–124)	52 (38–65)	260 (242–278)	91 (66–124)	80 (54–106)

The figures in parentheses indicate the extreme variations.

3 days, the increase in heart rate was only about 30 per minute. In our rats given 30 micrograms of thyroxine daily, that is to say about 2.1 micrograms per 10 grams of body weight, the heart rate exceeded that of thyroidectomized controls by more than 200 beats per minute. The effect on the heart of the

smallest dose of thyroxine used, namely, 3 micrograms (or 0.21 microgram per 10 grams of body weight per day) was quite clear, increasing the heart rate by 79 beats per minute. This was in marked contrast to the inactivity of thyronine, diiodotyrosine, and potassium iodide, and to the activity of diiodothyronine, which in a dose of 412 micrograms daily was not significantly more effective than 3 micrograms of thyroxine. Therefore, the effect of thyroxine on the heart appeared highly specific, and it seems superfluous to postulate the existence of other active compounds to explain the cardiotropic effect of the thyroid.

The results in table 1 show good agreement between the values for metabolic and cardiac rates, as far as the thyroid substances are concerned. In the case of dinitrophenol, however, there was a discrepancy between metabolic and cardiac stimulation. During periods of marked metabolic hyperactivity produced by this drug (table 2), the heart rate was either not influenced or only slightly affected. There was a clear-cut discrepancy between the variations in body temperature and breathing rate, which were directly related to the intensity of the metabolic stimulation, and the heart rate, which was hardly affected by considerable variations in metabolism. These results implied that the heart stimulation due to thyroxine or thyroid preparations was not solely the result of the general metabolic hyperactivity, but was probably due to a direct effect of these substances on the heart. That this is actually the case was indicated by the work of Markowitz and Yater (17), showing that thyroxine increased the rate of contraction of embryonic cardiac muscle in tissue culture.

The incidental observation that dinitrophenol was somewhat less effective on the metabolism in thyroidectomized than in normal rats (table 2) may be contrasted with the fact that much greater increase in metabolism is produced by thyroid hormone in thyroidectomized than in normal subjects (1, 18).

SUMMARY

1. Thyroidectomy decreases the heart rate of the rat from 431 to 339 beats per minute in fully conscious, restrained animals, and from 379 to 265 beats per minute in anesthetized animals, on the average. A dose of 3 micrograms of thyroxine daily restores the heart rate almost to normal, while doses of 30, 300 and 3000 micrograms give values above normal, reaching in the case of the highest dose 647 in conscious and 574 in nembutal-treated rats.

2. Diiodothyronine in a dose of 412 micrograms daily in thyroidectomized rats raises the heart rate to the normal range. Thyronine, diiodotyrosine, and potassium iodide appear to have no effect on the heart of the thyroidectomized rat.

3. In general, thyroidectomy reduces, and graded doses of thyroxine increase in a parallel fashion heart rate, body temperature, breathing rate, and oxygen consumption.

4. Dinitrophenol stimulates breathing rate and body temperature along with oxygen consumption, but it affects the heart rate only slightly. This discrepancy suggests that for the most part the effect of thyroxine on the heart rate

is not the consequence of general metabolic stimulation but is due to a direct effect on the heart.

5. Thyroxine appears to be the specific heart-stimulating agent in thyroid preparations.

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ON THE MECHANISM OF THE ADAPTATION OF PANCREATIC ENZYMES TO DIETARY COMPOSITION

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Received for publication November 1, 1943

It has been demonstrated (1) that in rats maintained on a constant diet an adaptation of the chief pancreatic enzymes to the predominant constituent of the diet occurs. Thus, a high carbohydrate diet produced a pronounced increase in the amylase content of the pancreatic tissue, a high protein diet resulted in greatly increased trypsin content, whereas a high fat diet caused no important alteration in lipase (table 1). It was further demonstrated in the dog that the relative concentration of the chief pancreatic enzymes in pancreatic juice parallels that existing in the pancreatic tissue.

Previous investigators of the problem of adaptation of pancreatic enzymes to the diet (literature cited in our previous paper (1)) have not considered the mechanism of this adaptation. A consideration of the possible mechanisms concerned suggests three possibilities: *one, a reflex secretory mechanism* in which, for example, increased protein in the intestine would reflexly stimulate the production of trypsinogen; *two, a hormone mechanism* in which, for example, increased protein in the intestine would cause a hormone to be formed which would specifically excite trypsinogen formation; and *three, a humoral mechanism* in which, for example, the digested products of protein in the blood stream would excite the formation of trypsinogen. Another question is also involved, namely, is it, for example, an excess of undigested protein or of partially or completely digested protein that is serving as a stimulus in the intestine or in the blood stream.

The following experiment was performed to provide information bearing on the foregoing questions.

METHODS. Thirty-seven young white rats (150–250 grams) served as experimental animals. The animals were divided into four groups, ten animals in each group, except for one group of seven. *Group one* was fed a balanced diet (table 2) and served as the control. *Group two* was fed a diet identical with the balanced diet except that the corn starch was replaced by an equivalent amount of dextrose. In the diet of *group three* casein hydrolysate (Amigen, Mead) was substituted for the casein of the balanced diet. *Group four* was fed the unaltered balanced diet and received a daily subcutaneous injection of two units of protamine zinc insulin (Iletin, Lilly). The diets were fed *ad libitum* for 21 days at the end of which time the animals were sacrificed by cervical fracture. The method for obtaining, preparing, and analyzing the pancreatic tissue was the same as that used by us previously (1).

RESULTS. When the enzyme content of the pancreases of the animals in groups 2 to 4 was compared with that of the animals maintained upon a balanced

diet, several deviations were noted. The dextrose diet produced an increase in the amylase and lipase content of the pancreatic tissue with no significant change in the trypsin. The animals fed the casein hydrolysate diet showed a decrease in trypsin content without significant change in lipase or amylase.

TABLE 1

Deviations from average enzyme composition of pancreatic tissue as affected by dietary composition

DIET	TRYPSIN	AMYLASE	LIPASE
Balanced	Average	Average	Average
High protein casein	Increased	Diminished	Increased
High carbohydrate Corn starch	Diminished	Increased	Average
High fat lard	Average	Diminished	Average

TABLE 2

Composition of balanced diet

CONSTITUENT	PER CENT BY WEIGHT	CALORIES	PER CENT OF TOTAL CALORIES
Casein	18	72	15
Starch	47	188	38
Salt mixture (Wesson)	4	0	0
Cellulose	2	0	0
Lard	18	162	33
Yeast powder	8	40	8
Fish liver oil	3	27	5

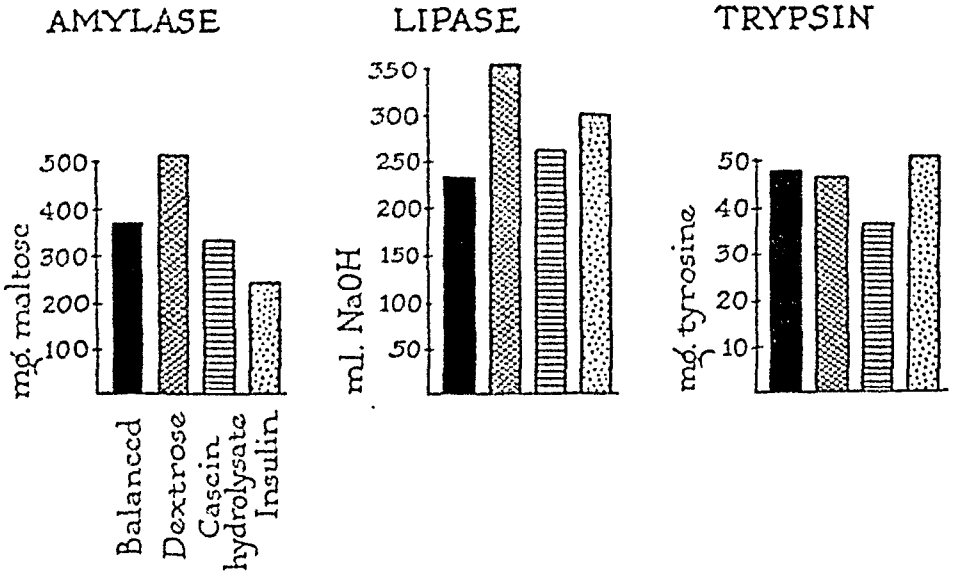

Fig. 1

The daily subcutaneous injection of protamine zinc insulin produced a repression of amylase, the lipase and trypsin remaining essentially unchanged. The mean values for each group are presented graphically in figure 1. The significance of the variations in enzyme values was tested by appropriate statistical analysis, the results of which appear in table 3.

Discussion. On the basis of the hypothesis that the substitution of the hydrolyzed form of a foodstuff for the unhydrolyzed form will determine whether

the presence of the undigested foodstuff in the gut is necessary for the elicitation of the characteristic pancreatic enzymic response, we may draw the following conclusions. In the case of starch, the product of its enzymatic digestion, dextrose, is capable of exciting an increase in amylase. Conversely, casein hydrolysate does not induce an augmentation of the trypsin content such as is noted with casein. This would suggest a nervous reflex factor for trypsin

TABLE 3

Statistical analysis of enzyme data indicating significance of deviations from values for balanced diets

DIET	MEAN	STANDARD ERROR OF MEAN	DIFFERENCE OF MEANS	STANDARD ERROR OF DIFFERENCE OF MEANS	CRITICAL RATIO "C"	PROBABILITY OF OCCURRENCE OF DEVIATION AS GREAT AS DESIGNATED "C"
Amylase						
Bal	379	±22.3				
Dextrose	509	±15.8	130	±27.4	4.8	0.0013%
Cas. Hyd	336	±16.9	43*	±28.0	1.6	10.96
Insulin	243	±24.0	136	±32.8	4.1	0.0057
Trypsin						
Bal	48.5	±4.7				
Dextrose	47.8	±1.7	0.7*	±4.95	0.14	50.0%
Cas. Hyd	37.4	±1.7	10.4	±4.96	2.1	3.57
Insulin	50.8	±1.6	2.3*	±4.95	0.46	50.0
Lipase						
Bal	240	±20.6				
Dextrose	342	±17.2	102	±26.8	3.8	0.0145%
Cas. Hyd	266	±19.6	26*	±28.4	0.9	36.81
Insulin	301	±30.0	61*	±36.4	1.7	8.9

* These differences are *not* significant.

regulation and a humoral agency involving dextrose for amylase. However, it is also possible that the failure of amino acid mixture to stimulate trypsin formation may indicate that a more complex hydrolytic product of protein, e.g., proteose, is the stimulus. The casein hydrolysate used in these experiments had 26 per cent of its nitrogen content in forms other than amino and ring nitrogen, probably representing imino and polypeptide nitrogen. No proteose was present in the casein hydrolysate.

In the case of amylase a humoral mechanism is further indicated by the repression of amylase produced by maintaining the blood sugar at a hypo-normal level by means of protamine zinc insulin.

A discrepancy was noted in the effect of corn starch and dextrose on lipase. The former caused no change whereas the latter produced a statistically significant increase in lipase content.

Evans (2) has demonstrated an augmentation in the amylolytic power of human saliva following the ingestion of carbohydrate. Only carbohydrate food elicits this response and mere chewing, without swallowing, is ineffective. Recently Harper and Vass(3) found that in cats the injection of foodstuffs into the duodenum resulted in an increase in enzyme output by the pancreas even when all the extrinsic nerves to the small intestine had been cut. Harper and Raper (4) have prepared an extract of the small intestine which stimulates the secretion of pancreatic enzymes without augmenting the volume rate of pancreatic juice. They have proposed the name "pancreozymin" for this substance and suggest that it may have physiological importance. Thus, several mechanisms for the special control of enzyme secretion are known. However it is unlikely that these mechanisms are concerned in the type of adaptation dealt with in this work because *a*, these mechanisms are instances of immediate alteration in enzyme secretion whereas the adaptation to diet here noted requires a prolonged period, and *b*, in the case of the pancreas, mechanisms now known to increase enzyme secretion cause a parallel increase in all the chief enzymes whereas the adaptation to diet involves the selective increase in the enzyme acting on the predominant constituent of the diet.

SUMMARY AND CONCLUSIONS

1. Substitution of dextrose for corn starch in a balanced diet results in increased amylase content of the pancreatic tissue. Administration of protamine zinc insulin to rats fed a balanced diet causes a depression of amylase content.

2. Substitution of casein hydrolysate for casein in a balanced diet produces a depression of trypsin content of the pancreatic tissue.

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ARTERIAL, CEREBROSPINAL AND VENOUS PRESSURES IN MAN DURING COUGH AND STRAIN[1]

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Received for publication November 2, 1943

It has been shown in scattered reports (1, 2, 3, 4) from this laboratory that sudden changes of intrathoracic and abdominal pressure such as occur in coughing and straining produce simultaneous changes in the arterial pressure and in cerebrospinal pressure. It was argued that whereas these pressure changes may strain the arteries of the arms, legs and skin, the more vital arteries of the thoracic and abdominal organs and of the central nervous system are protected against the extra strain made when the intrathoracic pressure goes up during a cough or strain.

The use of the differential manometer (5) has enabled us to show in a simple graphic form the differences in pressure which bear upon the peripheral and vital arteries and to differentiate the effects of direct pressure propagation from the secondary pressure changes resulting from the effects of intrathoracic pressure upon blood flow.

Technical difficulties make it hard to use the intrathoracic pressure itself to work a differential manometer. It is therefore assumed that the gross changes in the intrathoracic pressure are the same as those in the mouth when the lips and nose are closed and the glottis open. A sudden expulsive effort under these circumstances is thought to have the same effect on buccal and intrathoracic pressure and to simulate the effects of a cough upon the hemodynamics. A prolonged expiratory effort against the mouthpiece is proposed as having similar effects to a strain against a closed glottis as in a difficult bowel movement and breathing deeply through the mouthpiece against heavy resistance is thought to illustrate an exaggeration of the effects of breathing upon the blood pressure. On these assumptions the pressure relationships were recorded as follows.

The manometer is of the usual differential type as described elsewhere (5). It is a simple hypodermic manometer (1) whose moving parts are enclosed in an air tight chamber fronted with an optically plane glass plate. The manometer is thus constructed to measure the difference between pressures rather than pressure *per se*. The buccal pressure is led from a mouthpiece to the front chamber and the arterial pressure is led in the usual way to the manometer itself. The upper record in figures 1, 2 and 3 is from the differential manometer and measures roughly the excess of the arterial pressure over the intrathoracic pressure. That is to say it measures the pressure which strains on the walls of the intrathoracic and intra-abdominal arteries. The middle record is made by a manometer which is connected by a leaden T tube to the same arterial needle

[1] Aid from a grant by the American Medical Association is gratefully acknowledged.

as serves the differential manometer. It records unmodified the brachial pressure, i.e., the stresses on the arteries of the arms, legs and skin.

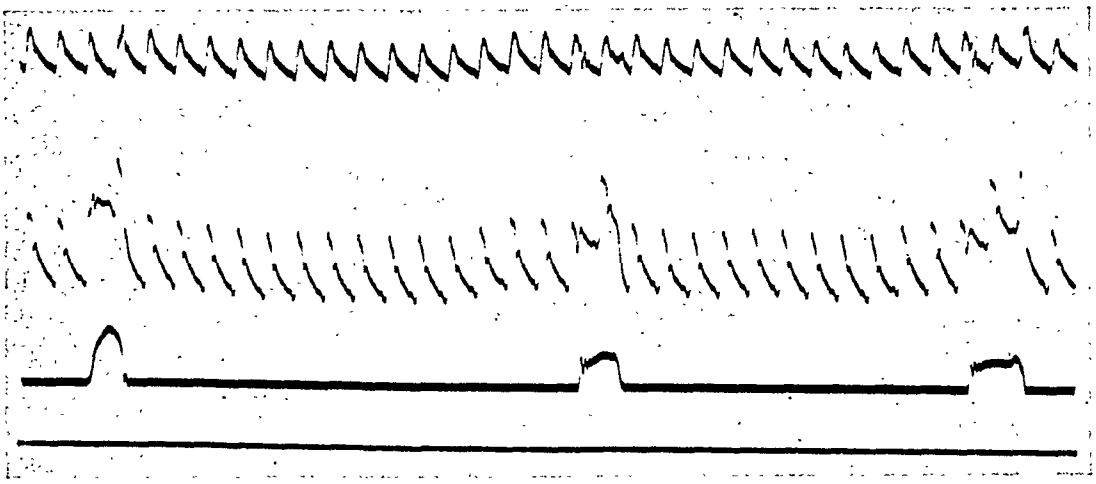

Fig. 1. Differential pressure record of "cough". The lower tracing is from a mouthpiece into which a forcible expiration was made. The next record is of the arterial pressure while the upper record is a differential record of the middle record minus the lower record. The mouthpiece record shows pressure changes nearly identical with intrathoracic pressure changes and the differential record indicates the stresses which the coronary and similar intrathoracic arteries undergo. The short increases in "intrathoracic" pressure are similar to those in a cough.

Fig. 2. Same during a prolonged strain

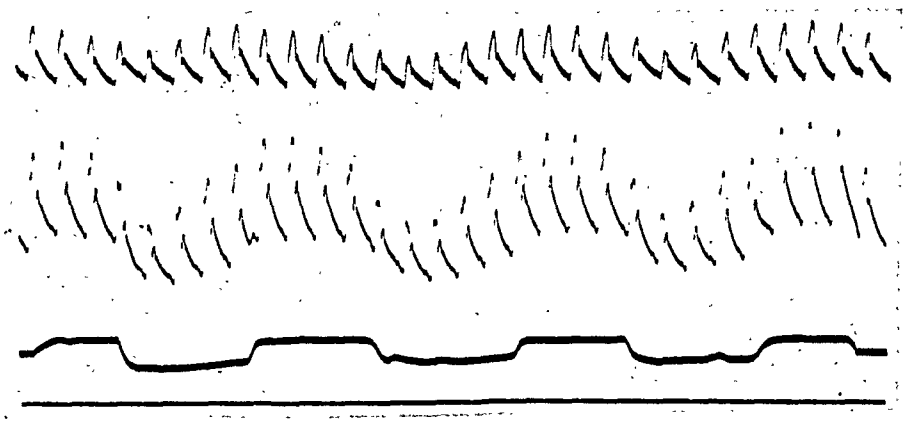

Fig. 3. Same during alternate inspirations and expirations

Figure 1 shows the effect of a short expiratory effort against the mouthpiece. The abrupt increases in the peripheral arterial pressure shown in the middle

record are cancelled out of the upper record by the fact that pressures recorded in the lower record are subtracted by the action of the differential manometer. This illustrates the fact that the vital arteries are protected against such stresses.

In figure 2 pressure is maintained within the thorax and abdomen long enough to hinder venous return. The arterial pressure shows a rise for as long as the strain lasts but the differential pressure (vital arteries) shows a simple fall with a decrease of pulse pressure. This is followed by an increase in both mean pressure and pulse pressure as the blood dammed back in the veins floods into the heart.

In case of figure 3 the subject was required to breath back and forth against heavy resistance. The arterial pressure showed much greater fluctuations than the differential pressure. Each increase in intrathoracic pressure is followed by a decrease in differential arterial pressure and each decrease in intrathoracic pressure, since it aspirates blood into the heart is followed—as soon as blood can get through the lungs—by an increase in the differential arterial pressure and in the pulse pressure.

It should be stressed that conditions obtaining in this experiment are far different from those obtaining during normal respiration. A man breathing naturally shows little or no respiratory changes in the pulse pressure. Particularly if he is recumbent the venous pressure is such that blood can get to the heart unhindered by the respiratory fluctuations in thoracic pressure. If the venous pressure is high enough so that the normal fluctuations in intrathoracic pressure do not affect cardiac inflow, then the respiratory changes in arterial pressure are simple propagations of intrathoracic pressure. Normally this is so but if breathing is against resistance such as accumulated mucus or laryngospasm, or if the venous pressure is abnormally low, the respiration can evoke changes in blood flow which give rise to the phenomena shown in the figure.

When a cough or strain occurs and the pressures in the thoracic and abdominal cavities rise the pressure bears upon the intervertebral foramina. This results in the movement of small amounts of material back into the cerebrospinal canal through the intervertebral foramina. The material which is forced into the canal includes the cerebrospinal fluid under the dural evaginations of the spinal roots, venous blood, loose fatty and areolar tissue as well as the spinal nerves themselves (6). The craniospinal canal is so rigid that only a minute amount of such substance need go into the canal to equalize the pressure to that in the thorax and abdomen during the cough or strain. The result is that the cerebrospinal pressure goes up immediately to a figure that is equal to that in the thorax and abdomen.

It is generally recognized that a rise in jugular pressure is accompanied by a rise in cerebrospinal pressure and that the arterial pressure is also closely related to the cerebrospinal pressure (7). The following observations show, however, that the intrathoracic pressure rise is not propagated to the cerebrospinal canal by either the arterial or venous channels. Figure 4 shows simultaneous records of internal jugular, spinal and intrathoracic pressure. The rise in jugular pressure lags behind the rise in both thoracic and spinal pressure and unless the

effort is prolonged the jugular pressure does not reach as high a figure as the spinal and thoracic pressure.

The rise in arterial pressure which accompanies the cough is also of no consequence in the transmission of the cough pressure to the cerebrospinal cavity. A rise of blood pressure of 40 mm. Hg accompanying an ordinary arterial pulsation has no counterpart in the cerebrospinal pressure while an arterial pressure rise of similar height but accompanying a cough is exactly reproduced in the spinal pressure (see fig. 5).

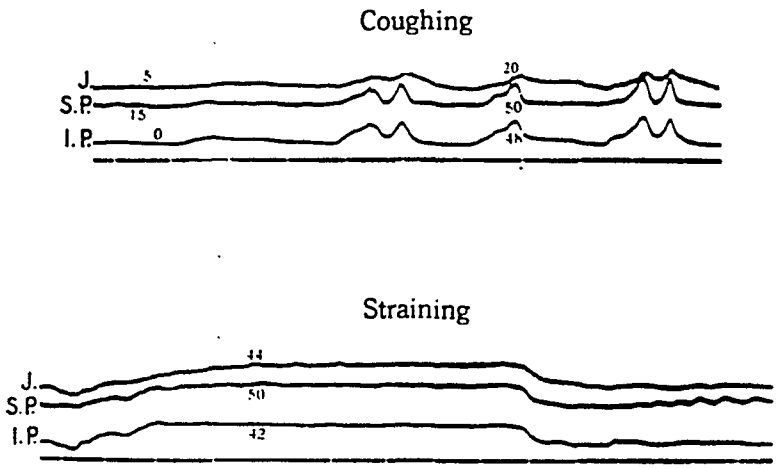

Fig. 4. Jugular, spinal and intrathoracic pressures during a cough and strain

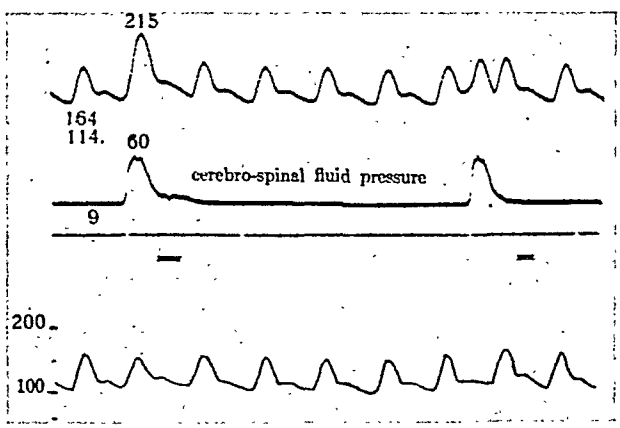

Fig. 5. Arterial pressure and cerebrospinal fluid pressure during two coughs. The lower record is a graphical reconstruction of the difference between the pressures recorded in the upper curves.

The fact that the cerebrospinal pressure has such small pulsations is rather puzzling in itself. Normally the contours show evidences of a systolic rise which is aborted by a fall simultaneous with the descent of the C wave of the venous pulse. The diastolic rise is not so evident in the cerebrospinal pulse as it is in the venous pulse. In short the contours of the cerebrospinal pulse indicate that it is normally a summation of arterial and venous pulsations and since these are essentially reciprocal the actual pressure pulse in the normal cerebrospinal canal is very small.

When the arterial pulsations are exaggerated as they are after the termination of the Valsalva experiment or in dogs after a dose of epinephrine the pressure pulses in the cerebrospinal canal become greater and assume the arterial contour, i.e., rise in systole and go down in diastole (1).

The opposite is true when the venous pressure is increased and the venous pulsations can be transmitted more strongly to the cerebrospinal canal. In cases of congestive failure the pressure pulses of the cerebrospinal canal are exaggerated but they have the venous contour, i.e., they rise in diastole and decline in systole (see fig. 6).

Figure 6 also illustrates another physiological phenomenon that is worth consideration. When the strain begins the intraspinal (intrathoracic) pressure rises and there is an equal rise of the blood pressure. In contrast to the normal there is no falling off of the blood pressure and the pulse pressure because venous pressure is so high that blood fills the heart in spite of the high intrathoracic pressure. When the strain is over the arterial pulsations go back to normal with

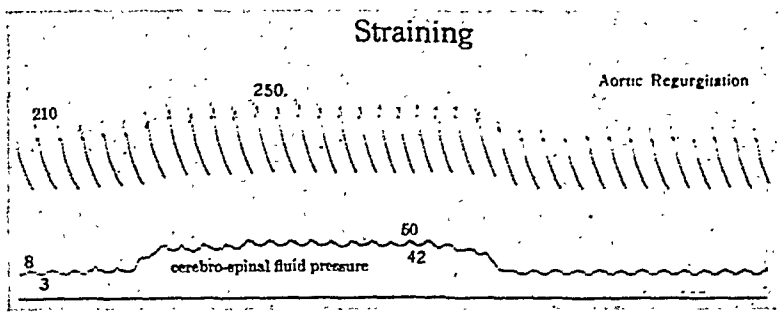

Fig. 6. Strain in a case of decompensated heart disease. Above, blood pressure; below, intraspinal pressure.

no compensatory increase in pressure such as is seen in normal individuals when the blood dammed out of the chest surges back in.

It has been shown (8) that reflex adjustment of heart rate to blood pressure changes is absent in dogs with aortic regurgitation. Figure 6, from a case of aortic regurgitation, indicates that the same may be true in man.

When, during the cough, the effective arterial pressure increases in the periphery there is an increase in the arteriolar run off in all beds outside the thorax, abdomen and cerebrospinal canal. This has a striking effect in certain cases with hypodynamic circulation, upon the net aortic blood pressure as is seen in figure 7, *A*. The aorta and its branches are emptied of blood through the increased peripheral drainage and the net pressure within them goes down very rapidly. During the rise of intrathoracic pressure (measured directly from a pneumothorax) it descends to 10 mm. Hg. Later when this same individual had a paroxysm of coughing, successive coughs reduced the net aortic pressure successively more quickly and more extensively until it reached the value of zero. On occasions the pressure remains below 20 mm. Hg for most of diastole and since the heart gains much of its blood during diastole it probably has its blood supply restricted during the whole of a cardiac cycle. When the effective aortic

pressure is zero and the peripheral arterial pressure is maintained by the intrathoracic pressure only, there is no head of pressure to irrigate the coronary or other beds which are entirely within the thoracic, abdominal or cerebrospinal cavity.

This situation does not obtain in individuals whose circulation is normal. Careful measurements have been made in eight normal subjects. The net aortic pressure went down during the cough in nearly all cases but it did not go down more than a few millimeters because the peripheral resistance was not abnormally low.

Fortunately the lowering of the effective aortic pressure is only temporary. The high pressure phase of the cough is followed by the expulsive phase. During this phase the effective aortic pressure will rise even during diastole. The rise can be shown only in cases with hypodynamic circulation and in cases of congestive heart failure. This phenomenon is illustrated in figure 7, *B*. During

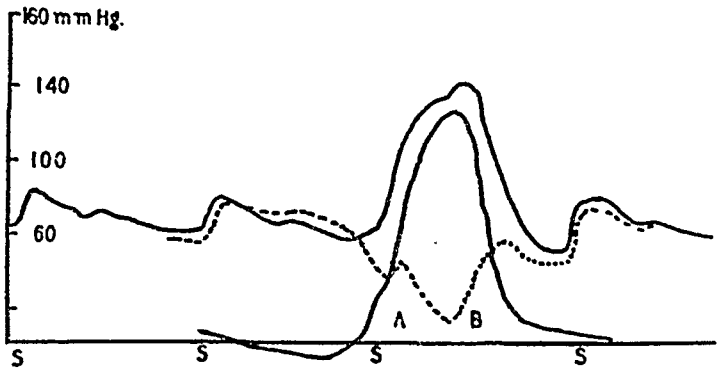

Fig. 7. Cough in case of tuberculosis with hypodynamic circulation. Above arterial pressure, below intrathoracic pressure measured directly from a pneumothorax. The dotted line gives the difference between the two, i.e., the net aortic pressure. During *A* the net pressure is being reduced to 10 mm. Hg. During *B* the net aortic pressure rises during diastole. *S* indicates the beginning of systole.

the expulsive stage of the cough, which may begin considerably before the peak of the intrathoracic pressure curve (1) the lungs are being forcibly emptied of air. The same effort that empties them of air also empties them of blood. It has been shown (9, 10, 11, 12) that the capacity for blood is less when the lungs are deflated than when they are distended. If then a forcible expiration can expel blood from the lungs with sufficient pressure and if the pulmonary valve is competent the pulmonary blood will necessarily push open the mitral and aortic valves and enter the aorta. It is difficult to explain the diastolic rise in net aortic pressure in any other way. The rise as we have reconstructed it comes from the fact that the brachial pressure may continue to rise during the expiratory phase of the cough. When not actually rising the arterial pressure often falls less rapidly than the intrathoracic pressure. Pressure energies which are dissipated in moving the lung tissues or the lung air are a part of the thoracic pressure curve and hence are subtracted from the blood pressure in making the construction of net aortic pressure. A disproportionate rise in this factor would

tend to lower rather than raise the net aortic pressure. The rise in net arterial pressure cannot be explained by the fact that transmission of pulsations over arteries is slow because the contours of the surges appear almost the same whether a time interval for this factor is or is not allowed in the construction.

An increased abdominal pressure acting upon the abdominal arteries will probably increase the general blood pressure but not to as great an extent as the combined action of a simultaneous increase of both thoracic and abdominal pressure. If the abdominal pressure exceeds the thoracic pressure during the cough some of the excess arterial pressure recorded could be due to excess abdominal pressure. Since hydrostatic differences were cancelled by the fact that the subject was recumbent, the important factors which might raise the abdominal pressure above the thoracic pressure are 1, inertia of the viscera; 2, tension on the relaxed diaphragm; 3, contraction of the diaphragm. Rough calculations would indicate that the first factor would involve pressures less than 2 mm. Hg. The second factor might operate to some extent as the lungs are finally emptied of air and since the expulsive stage of the cough is free of inspiratory efforts it is probable that the diaphragm does not contract. Although we have no records of simultaneous abdominal and thoracic pressure, we feel that the abdominal pressure does not rise above the thoracic pressure during the cough enough to account for the excess arterial pressure.

If the expiratory effort drives blood out of the lungs and into the aorta against the systemic pressure a very high total pressure must exist in the pulmonary veins. These veins are without valves and the pressure must be backed up as far as the semilunar valve, distending the capillaries. Both sets of cardiac valves on the left side must be open and the heart be filling the pericardium.

The pressure which can push blood out into the aorta is the pressure distending the pulmonary vein minus the net aortic pressure. Since the first stage of the cough depleted the aortic blood in individuals with a hypodynamic circulation (fig. 7 *A*) the net aortic pressure is very low and blood is free to move from the lung vessels through the left heart and into the aorta. This it does until the pressure in the aorta equals the sum of the pressures in the thorax and pulmonary vessels, raising the aortic pressure in some cases to a figure that is 30 to 60 mm. Hg above the thoracic pressure. This difference must be the pressure in the pulmonary vein during the expulsive effort. This figure seems high but it is no higher than that seen after epinephrine (9). Its effect on the net aortic pressure is made evident by the fact that the pressure is rising during diastole. When the pressure is falling the aortic valve may be closed and the pulmonary venous pressure may be less than the net aortic pressure. This may also be true during systole when the mitral valve is closed.

The low net aortic pressure which occurs in individuals with hypodynamic circulation favors the movement of blood into the aorta. A high pulmonary venous pressure would be expected to do the same thing. When the net aortic pressure was plotted during the expulsive stages of coughing in a case of congestive heart failure a diastolic surge of pressure similar to that shown in figure 5 *B* was evident on several occasions. The excess of aortic pressure over intra-

thoracic pressure was 60 to 80 mm. Hg. This is probably the pulmonary venous pressure obtaining at the time.

The pressure which acts upon the walls of the alveolar capillaries is probably much greater than the figure above because the air pressure in the alveoli can hardly be as high as the intrathoracic pressure. During the expiratory phase of a cough the energy which is present in the high intrapleural pressure is available to move the lungs, to accelerate air out of the bronchial tree and to accelerate blood out of the pulmonary vascular tree.

There is no way of measuring what part of the expiratory intrathoracic pressure energy is dissipated in moving blood, what part in moving lung tissue and what part in moving lung air. On the guess that the energy used in moving lung air and tissue is a relatively small fraction of the total it follows that the expiration produces a small rise in air pressure in the alveoli. This pressure change cannot be measured but circumstances point to the idea that during the expiratory phase of the cough the pressure in the pulmonary capillaries which is sufficient to drive blood into the systemic arteries is probably far above the pressure of air in the alveoli. High pressure across the capillary walls must result in filtration of fluid into the alveoli. Paroxysmal coughing therefore may clear froth from the bronchioles and alveoli but it also establishes a vicious cycle which results in the transfer of more fluid into these spaces and the further stimulation of the cough reflex. The fact that coughing raises the pressure in the pulmonary vascular tree in addition to the mechanical movements involved increases the risk of pulmonary hemorrhage during this activity.

If the circulation is normal and the lungs not at all congested, the coughing does not, as has already been said, produce an appreciable surge of blood into the arteries. It is probable, however, that violent coughing does increase the capillary pressure in the lungs and does exaggerate the tendency of fluid to accumulate in the alveoli. The physiological picture thus outlined may, when it is recognized, help to decide in an individual case whether coughing should be encouraged for the purpose of clearing the lungs or whether it should be repressed with sedatives to keep down the transudation of fluid into the alveoli as well as for other well recognized purposes.

SUMMARY

1. Differential pressure records are shown which separate the changes in arterial pressure which are due to simple propagation of intrathoracic pressure from those which are due to changes in blood flow. It is shown that increases due to the first of the above causes strain only the peripheral arteries whereas increases due to changes in blood flow or to changes in peripheral resistance strain also the vital arteries to the brain, spinal cord and viscera.

2. The nature of the cerebrospinal pressure pulsations is discussed.

3. During the preliminary pressure rise of the cough people whose circulation is hypodynamic show arterial pressures which are no higher than simultaneous intrathoracic pressures. During brief intervals there is therefore no effective head of pressure to irrigate the coronary or other vital vascular beds.

4. During the expulsive phase of the cough the arterial pressure may continue to rise while the intrathoracic pressure is going down or the arterial pressure may descend more slowly than the intrathoracic pressure. This signifies that the pressure distending the aorta is rising and, since it often occurs during diastole, it implies that during the expulsive phase of a cough blood is forced from the lungs through the relaxed left heart and into the aorta.

5. The cough may force blood into the aorta in cases with hypodynamic circulation and in cases with congestive heart failure. This may occur in normal individuals but no evidence has been obtained to support the idea that it does.

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CHANGES IN THE VITAL CAPACITY WHEN THE BODY IS IMMERSED IN WATER

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Received for publication November 15, 1943

It is generally accepted that when one is recumbent the vital capacity is significantly less than when he is standing. This might be due to the fact that the respiratory muscles work at a disadvantage in moving the viscera or ribs in the recumbent position or that the recumbent position favored movement of blood back to the heart whence it was pumped into the lungs. If the systemic circuit demands less blood than can return to the heart in the recumbent position there should be a backing up of blood in the lungs. That this is so is indicated by two things. Measurements of the chest by x-ray at full inspiration and at full expiration do not indicate a postural difference in the extent of the respiratory excursion (1) and secondly, hindrance of venous return by means of blood pressure cuffs around the bases of the four extremities and blown up to 70 mm. Hg caused the vital capacity in the recumbent posture to approach the figure when the subject was standing (1, 2).

These observations lead to the conclusion that circulatory factors play an important rôle in the postural reduction of vital capacity. Another possible way to bring out the circulatory factors in the control of vital capacity is to explore the effects of immersing the body in water. When the body is immersed in water the water presses through the flesh upon the veins. This pressure is greater on those parts that are more deeply immersed balancing to a great degree the hydrostatic pressure made by the venous column. This means that the blood floats back to the heart and need not be forced back when the subject is standing any more than when he is horizontal. In addition, immersing the body neutralizes the weight of the viscera which to a minor extent may handicap the expiratory effort. The inspiratory effort will of course have to work against the weight of the water, counterbalancing the effect of the water weight upon expiration and making the mechanical movements as free under water as above.

When the subjects (20 male medical students) stood in water up to the nipple line, the vital capacity averaged 4548 cc. Upright and out of water the vital capacity was 4863. When cuffs were applied to the bases of the four extremities and blown up to 70 mm. Hg the vital capacity increased from 4548 cc. to 4721 cc. The difference between these two figures is well beyond random variation because the individual data all showed a decrease when the subject went from air to water and all but one an increase when the cuffs were applied to the subject while he was in water. Each figure in table 1 represents the average of ten or more observations.

There is also a statistically significant difference between the figure representing the average vital capacity in water with cuffs and that representing the vital capacity standing in air. (P. is better than 0.01.)

The fact that the cuffs do not enable a man to breathe as much air while in water as he can standing on the platform indicates either that venous return is less when standing dry or that standing to nipple height in water offers some mechanical hindrance to the respiratory movement. It has been argued above and elsewhere (1) that neither immersing the body nor assuming the recumbent posture offers any important mechanical hindrance to the respiratory movements. Immersing the body or recumbency increases venous return from the lower trunk as well as from the arms and legs. The cuffs reduce return from the arms and legs but leave unaffected the gravitation effects on venous return produced by either posture or immersion on the vessels of the trunk and this increase in venous return may be enough to explain the change in vital capacity.

TABLE 1

Effect of immersing body in water upon the vital capacity with and without hindrance to the venous return

	OUT OF WATER	IN WATER			OUT OF WATER	IN WATER	
	Withouts cuffs	Without cuffs	With cuffs		Without cuffs	Without cuffs	With cuffs
	1	2	3		1	2	3
RJB	5610	5330	5780	HJB	4760	4260	4550
GES	4160	3960	3820	CCB	4670	4180	4340
WO	5230	4750	4990	LB	4490	3990	4280
ZEG	3980	3570	3730	APP	4920	4700	4770
JPM	5090	4760	4910	GC	5280	5100	5230
WLS	3750	3440	3610	MME	4510	4390	4850
LP	5030	4670	4910	EJM	6230	5750	6000
HC	4270	3820	4030	BD	5020	4710	4950
LH	4540	4190	4260	MM	5290	4600	4990
JTH	4500	4260	4330	GO	5930	5530	5880
Averages.....					4863	4548	4721

Certain additional observations are of interest. When the subject lies supine in water his vital capacity is no greater than when he stands upright in water. If he is allowed to settle as he forces the air out of his lungs, completing the expiration at a depth of two or three feet, the added pressure on the thorax enables him to expel a larger than normal "vital capacity." These experiments were performed with a nose clip firmly in place and a mouthpiece connected to the spirometer by a long tube. When the subject had settled under the water with the understanding that he was to inhale as soon as he had exhaled completely he found that it was nearly impossible to make the effort. This was not because he was so deep under water that he could not accomplish the muscular act of expanding the chest but was due to stimulation by the water on the face or some similar factor that prevented his even trying to inhale. The situation seems to be analogous to the more highly developed protective reflexes in diving animals

which inhibits breathing when water covers the face and even results in asphyxia if a drop or two gets into the nostril (3). The force of the inhibition in man is not very strong because it may be broken through by a firm effort of the will. Breathing through a tube, with the face under water, does not seem natural but can be accomplished without too much effort after a few minutes' practice.

SUMMARY

The vital capacity is about 300 cc. less when the body is immersed in water to the nipple line than it is when the subject is standing in air. When diastolic pressure cuffs are put around the bases of the arms and legs of the subject in water the vital capacity is about 175 cc. higher than without the cuffs.

Indications are that the venous return is increased by immersion sufficiently to increase the load of blood in the lungs and decrease the space available for air.

Grateful acknowledgment is made to the Augusta (Ga.) YMCA and to Mr. W. H. Adkins, its Physical Director, for the use of its pool in carrying out these experiments; and to the many students of the University of Georgia School of Medicine who served as the subjects.

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EXPERIMENTAL ANALYSIS OF THE NERVOUS FACTOR IN SHOCK INDUCED BY MUSCLE TRAUMA IN NORMAL DOGS[1]

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Received for publication November 16, 1943

In a previous study in which shock was induced in dogs by traumatization of all faces of both thighs with a 200 gram rawhide mallet, it was observed that either prolonged spinal anesthesia, or thorough infiltration of the skin and muscles of the thighs with a 4 per cent procaine solution, administered prior to the traumatization and maintained for a period of 3½ hours, prevented shock in a large proportion of cases (1).

This evidence for the presence of an important nervous factor in shock following the type of leg muscle trauma employed would seem to be contradictory to earlier findings (2–6) that complete cord section did not prevent fatal shock following muscle trauma, and that severing the major nerves of the legs to be traumatized did not modify the shock picture. It seemed that the discrepancy between the protective effect of spinal anesthesia against shock, as opposed to the absence of effect of cord section, might lie in the fact that the animal subjected to spinal cord section previous to leg muscle trauma experienced much more drastic treatment than did the animal receiving only spinal anesthesia or local procaine treatment of the legs previous to trauma. Yet it has been observed by others (3–5), and confirmed by us, that chronic spinal animals develop shock following leg trauma just as do those in which trauma follows immediately after the cord section. It was apparent that since neither cord nor leg nerve section could protect against shock, elimination of the flow of afferent nociceptive stimuli was but one aspect of the situation in so far as nervous factors in shock are concerned.

A. *Control series.* A total of 37 healthy dogs have been subjected to leg muscle trauma of the type described by Solandt and Best (2), modified by Gregersen (3), and adopted by us (1). Deep ether anesthesia was maintained during traumatization and the animals allowed to rest on their backs in cradles for a period of six hours before returning to the cages. In these experiments the dogs were given water after 24 hours. Fifteen of the animals were done at an earlier date (1), the others were used as additional controls for the experiments to be

[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Princeton University.

[2] Some of the data included in this paper were taken from a thesis presented by William Kleinberg to the Graduate School of Princeton University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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reported here. Of these 37 animals, 33 or 89 per cent died in shock 4–9 hours after completion of the trauma. Blood pressure and pulse changes were similar to those described for the earlier control series (1) (fig. 3, I and table 2).

Plasma volume changes during the course of the experiment were determined by the dye T-1824 technique (7) on seven animals of this second control series.

TABLE 1

Plasma volume changes in dogs subjected to trauma to both hind legs

DOG NO.	INITIAL		AFTER TRAUMA		3 HOURS AFTER TRAUMA		PER CENT DECLINE IN PLASMA VOLUME
	Blood pressure	Plasma volume	Blood pressure	Plasma volume	Blood pressure	Plasma Volume	
Controls							
	mm. Hg	*cc./kgm. body weight*	*mm. Hg*	*cc./kgm. body weight*	*mm. hg*	*cc./kgm. body weight*	
1	131	51.5	69	37.9	78	34.2	33.9
2	133	47.5	68	41.1	23	36.2	23.8
3	131	54.0	58	36.7	0		
4	118	52.5	68	47.6	78	38.7	26.3
5	126	50.2	69	35.3	76	31.3	37.6
6	95	52.7	68	40.0	51	35.9	30.0
7	105	50.1	62	39.5	74	34.2	31.8
Ave.....	120	51.2	66	39.7	54	34.9	30.6
*Procaine anesthetization of leg nerves, standard amount of trauma**							
8	140	56.1	37		56	37.5	33.2
9	106	57.8	62		60	33.9	41.2
10	106	52.1	69		70	36.4	30.2
11	124	53.6	69		74	32.7	38.7
12	133	49.3	67		60	27.3	44.8
Extra amounts of trauma*							
13	150	51.4	68		74	22.7	55.8
14	132	55.4	70		88	26.1	53.3
15	120	50.0	60		65	25.0	50.0
Ave.....	126	53.2	63		68	30.2	43.4

* See text.

The direction and magnitude of the changes approximated those observed by others (3) in animals similarly shocked. The major part of the plasma volume fall occurred during the trauma or very shortly afterward (table 1), and presumably represented the pooling of blood in the injured area. Although the legs did not appear to swell markedly, considerable fluid and stagnant whole blood oozed through cut surfaces upon autopsy. The plasma volume continued to

decline so that a 31 per cent reduction was observed shortly before death of the animal.

It was pointed out previously (1) that hematocrit, hemoglobin and serum protein concentrations do not deviate significantly during the course of the experiment. There is, therefore, little evidence for preferential transudation of

TABLE 2

The effect of complete and partial spinal cord section, and of leg nerve section and anesthesia, on the responses of dogs to trauma to both hind legs

SERIES	EXPERIMENT	NUMBER OF DOGS	BODY WEIGHT	TOTAL NO. BLOWS	BLOWS/KGM. BODY WEIGHT	SURVIVAL PERIOD	NO. OF DOGS SURVIVED	PER CENT SURVIVED
			kgm.			*hrs.*		
A	Controls	37	12.2	1010	82	5 ±0.2	4	11
B	Complete spinal section	17	11.8	1040	88	14 ±1.8	1	6
C	Partial spinal section:*							
	C and D areas intact	18	11.7	1065	91	9 ±1.0	1	6
D	C and D areas cut	14	12.8	1006	79	11 ±1.1	2	14
E	C area cut, D area intact	34	12.1	1085	90	Indef.	29	85
F	Leg nerve section	12	11.2	970	89	7 ±2.0	1	8
G_1	Leg nerve anesthesia:†							
	Nerves exposed	10	11.7	1170	100	10 ±2.0	5	50
G_2	Nerves not exposed	11	13.0	1150	89	Indef.	10	91
G_3	Nerves not exposed, spinal section after trauma	9	8.2	1084	132	10 ±1.6	1	11

Blood pressure (mm. Hg) and pulse per minute

SERIES	INITIAL		AFTER OPERATION		AFTER TRAUMA		1–3 HOURS		5–6 HOURS		24 HOURS	
	Press.	Pulse	Press.	Pulse	Press.	Pulse	Press.	Pulse	Press.	Pulse	Press.	Pulse
A	120	158			62	150	82	175	45	195		
B	120	170	106	158	63	152	87	175	47	214		
C	112	163	97	148	58	161	85	190	43	220		
D	110	166	92	155	62	153	89	178	77	213		
E	114	167	105	162	61	155	86	189	87	188	110	146
F	113	160	106	149	62	170	75	190	57	227		
G_1	112	148	95	151	65	145	92	191	98	183	113	160
G_2	118	159	110	172	65	149	95	175	102	176	110	145
G_3	100	138			60	131	75	148	58	166		

* See figure 3 for spinal cord areas.

† See text for description of techniques.

plasma, and more for a pooling of whole blood in the vessels and tissues of the traumatized areas.

B. *Complete spinal section at the level of the last thoracic vertebra.* A total of 17 dogs were subjected to complete cord section. Thirteen were traumatized immediately after sectioning, and of this number 12 died and but 1 survived. The average survival period was 14 hours. In confirmation of the reports of others (3, 5), this failure of complete cord section to prevent shock is not to be

attributed to the trauma involved in the spinal operation itself, since four more chronic spinal animals, subjected to cord section 3 days previous to the experiment, also died in shock following the muscle trauma. Thus but one dog of the 17 survived cord section plus trauma (fig. 3, II).

Most of the animals in the acute spinal section series showed a moderate decline in mean arterial pressure after the operation. In only 3 cases, however, was this pressure level below 100 mm. Hg. More trauma was required to lower the pressure level to 60–70 mm. Hg by the end of trauma than for the control animals with cord intact (table 2). The development of the symptoms of shock, with falling pressure and high pulse rate was somewhat slower than in control animals, and the survival period was significantly longer (table 2, fig. 1).

It should perhaps be emphasized that all non-traumatized, spinal section animals survive the operation in excellent fashion. Hence the cord section it-

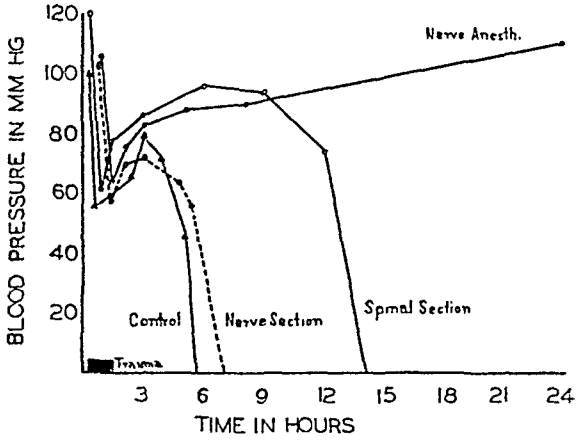


Fig. 1

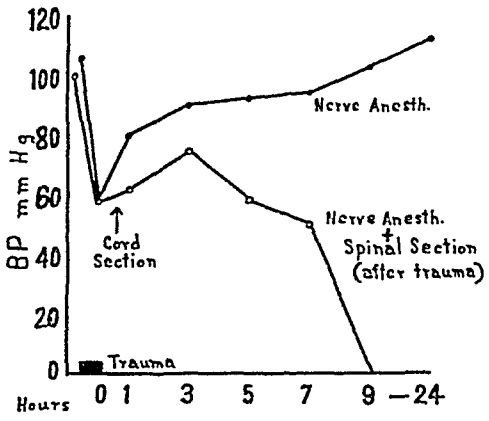


Fig. 2

Fig. 1. Blood pressure responses of representative cases from the control, leg nerve section, spinal cord section and leg nerve anesthetization series.

Fig. 2. Reversal of protective effect of leg nerve anesthesia when the spinal cord is sectioned immediately following trauma.

self did not lead to shock, nor in our experience was it ever fatal. When trauma to the legs was administered, however, shock developed and death followed.

C. *Section of the major nerves of the hind limbs followed by trauma.* Several workers (2, 4–6) have reported that section of the major nerves to the traumatized limbs does not prevent or appear to modify in any way, shock produced by muscle trauma. The following experiments confirm their findings.

The dogs were etherized and the hair removed from the upper portion of the legs with electric clippers followed by shaving. The skin was incised directly over the region where the external cutaneous, anterior crural, obturator and greater and lesser sciatic nerves emerge into the legs. By blunt dissection the nerves were exposed and severed anterior to the region to be traumatized. Section of the nerves necessitated four separate incisions in each leg. The small wounds were closed with clips and kept moist with gauze pads wet with chlorazene. Some blood continued to ooze from the wounds after traumatization.

Of 12 dogs so treated and then traumatized, 11 died and 1 survived. Hence severing the major leg nerves did not lead to prolongation of the life span nor alleviate shock in any way. The essential data are given in table 2 and figure 1.

D. *Effect of prolonged anesthesia of the major nerves of the hind limbs on prevention of shock following muscle trauma.* In work mentioned earlier (1) we showed that a 4 per cent procaine solution thoroughly infiltrated into the skin and muscles of all faces of the thigh area, previous to trauma, repeated at 30 to 40 minute intervals for 3½ hours, prevented shock in 7 of 10 animals. Since these results obtained by means of local anesthetization seemed diametrically opposed to those observed when the major leg nerves were sectioned, it seemed important to repeat and expand the previous work, this time restricting the local anesthesia to the region of the nerves themselves.

In a series of preliminary experiments, in which groups of 10 dogs each were used, various dosage levels of procaine were tested. In all cases injections were made at half hour intervals, and continued over a period of 3½ hours. The external cutaneous nerves of both legs were cut, the remaining major leg nerves were exposed as for section, and the procaine injected into and around the nerve sheaths. The best series was obtained with use of a 2 per cent procaine solution, where 5 dogs of 10 survived, and 3 more showed greatly extended survival periods. The major objection to the experimental technique was that, following trauma, considerable blood oozed through the eight cuts which had been made on the two legs to expose the nerves, and which were reopened at 30 minute periods in order to administer procaine.

The method was therefore modified so that in a final series of 11 anesthetized dogs, the procaine was injected through the skin and muscles into the vicinity of the major nerves without dissection and exposure of the latter. To insure a thorough anesthetization prior to trauma, a total of 2.5 to 3.5 cc. of a 4 per cent procaine solution was used for each dog, the exact amount depending upon the body weight. With subsequent injections, the volume of procaine was progressively reduced, so that the last injections totaled only 1 cc. distributed over both legs. A total of about 8 cc. of the procaine solution was used per dog over the 3½ hour period.

Ten animals of this series of 11 survived, and the life span of the single failure was prolonged to 20 hours. It should again be emphasized that throughout this study, no water was given until after 24 hours. Blood volumes were not determined.

There were several characteristic differences in response to the muscle trauma procedure between the animals given local injections of procaine and those either untreated or with leg nerves cut. About half the animals receiving procaine required significantly more trauma to lower the blood pressure to 60–70 mm. Hg. In some cases, as many as 50 per cent more blows were required than with a control of comparable size and body weight (fig. 1).

Plasma volume determinations were made on an additional 8 animals given local procaine anesthesia (table 1). Of these, 5 requiring standard amounts of trauma (i.e., sufficient to lower the mean arterial pressure in the femoral artery to 60–70 mm. Hg in a control animal of similar body weight) showed plasma volume reductions in the range of those observed for controls; 3 dogs which required more

than the usual amount of trauma to reduce the blood pressure, showed much greater plasma volume reduction by the same time interval (table 1).

A characteristic response of the traumatized procained animal is that the blood pressure tended to return much more promptly to high levels following the conclusion of the trauma. By the first hour the average pressure had reached 92 mm. Hg and it rose progressively from this level in the next few hours. In the series of animals which received procaine injections through the skin without preliminary dissection, 8 of the 11 animals showed pressure levels over 100 mm. Hg by the fifth hour after trauma (table 2).

It seems paradoxical that while anesthetization of the major nerves of the legs, when properly done, effectively prevented fatal shock in 10 of 11 cases, yet complete section of the same nerves was almost without effect in shock prevention. Both procedures should have eliminated the flow of nociceptive stimuli from the injured areas to the higher centers. It would seem, therefore, that nerve section, unlike nerve anesthesia, had permanently removed some nervous factor tending to maintain the resistance of the animal to shock induced by muscle trauma. To obtain more specific information regarding the site of this second, unsuspected, factor, the lack of which apparently sensitizes the traumatized dog to shock, a detailed physiological dissection of the spinal cord was undertaken.

E. *Destruction of spinal cord areas by means of knife, cautery and ligature.* In order to simplify matters, and if possible determine more precisely which ascending tracts might be involved in causing shock, the cord was, for purposes of surgical manipulation, arbitrarily marked off into areas, designated A, B, C, and D. None of these areas exactly correspond with the cord funiculi (fig. 3), but because their borders are defined by recognizable land marks, they can be identified on gross observation.

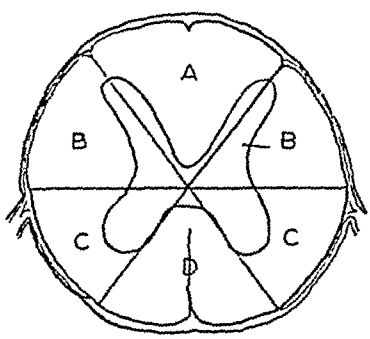
With the animal deeply anesthetized with ether, the spinal cord was exposed for a half-inch at the level of T 13 and L 1, care being taken to avoid all blood vessels. The dural sheath was opened and the selected area or areas, as depicted in figure 3, removed by means of 1, a fine blade knife; 2, a delicate tipped eye cautery, or 3, fine thread ligatures inserted by delicate needles. The attempted removal of a definite restricted region of the cord presents obvious difficulties. We have not found it possible with any of the three methods exactly to circumscribe in every case each area removed at operation, so that small portions of adjacent areas were sometimes injured. In most cases the grey matter was either partially or completely destroyed. The fine tipped cautery proved to be the most effective instrument for removing portions of the cord but had the disadvantage of inability of the operator to control the exact area of injury owing to "heat spread" from the cautery tip to adjacent areas not intended for inclusion in the area to be cauterized. Since the results obtained with the various methods have not proved different, they have been grouped together for analysis (fig. 3).

The cords of all animals were removed at autopsy, fixed in formalin, and examined under a microscope following free hand section with a razor blade through the injured areas. It is possible by this simple method to define fairly accurately the limits of injury.

Following the cord injury, the wound was closed except for a small gauze

drain, and the hind legs immediately traumatized as in the other experiments. The data obtained from a study of 83 dogs subjected to complete section or removal of some part of the spinal cord just previous to traumatization, are given in table 2 and figure 3.

In general, the results indicate that cord areas A and B are apparently not important for shock prevention or alleviation, for when they are destroyed and the animals traumatized, shock occurs. Of 18 dogs with areas A and B removed followed by traumatization, but 1 animal survived and 17 died (fig. 3, IV). The most important area for shock prevention appears to be the ventrolateral or C area, which must be removed bilaterally. When this area was completely removed, with the ventral D area left intact, 29 of 34 traumatized dogs, or 85 per cent, did not develop shock (table 2 and fig. 3, III and V). When the area C +



	CORD AREAS DESTROYED	DOGS SURVIVED	DOGS DIED IN SHOCK	TOTAL DOGS	PERCENT SURVIVED	SURVIVAL PERIOD, HOURS
I	NONE	4	33	37	11	5
II	ABCD	1	16	17	6	14
III	ABC	19	2	21	90	-
IV	AB	1	17	18	6	12
V	BC	10	3	13	77	-
VI	CD	2	12	14	14	14

Fig. 3. Arbitrary division of the spinal cord into areas, and the results of selective removal of these areas in preventing shock following leg trauma.

D was left intact, but one animal of a series of 18 survived after trauma (fig. 3, IV). Thus in the combinations tested, leaving C area uninjured is followed by fatal shock after muscle trauma.

It is also clear that area D, or the ventral cord region, must be left intact after eliminating area C, if the animal is to survive after leg trauma. Section of this area, as did complete spinal section, sensitized the animal to shock (fig. 3, VI). But 2 dogs out of 14 survived trauma when D area was destroyed (fig. 3, VI) and conversely, but 2 animals of a total of 21 died following trauma after removal of all parts of the cord except D (fig. 3, III). The evidence seems clear, in-so-far as these experiments on the cord are concerned, that in order to prevent shock after leg muscle trauma of the type described here, cord area C must be destroyed on both sides but area D left uninjured.

F. *Local anesthesia of the limb nerves followed by trauma and cord section.* In this experiment the spinal cords of 9 dogs were exposed as for section but left untouched, and the wounds then closed by clips. A thorough procaine infiltration of all faces of the thigh muscles was then made, using 4 per cent procaine, injected half hourly over a 3½ hour period, and the legs then traumatized. This series was therefore similar to that described above, where shock was prevented in 10 of 11 dogs (table 2). Immediately after the trauma, however, the incisions over the spinal cords were reopened, and the cords were completely sectioned[4]. But one animal of this series survived, with the survival period of the other 8 averaging 10 hours. Hence the spinal cord section negated the effect of the procaine infiltration and rendered the animal again sensitive to shock. The experiment emphasizes the importance of cord area D for maintaining normal resistance to leg trauma since destruction of this area and its descending tracts is apparently responsible for the sensitization to limb trauma which follows complete cord section (fig. 2).

Discussion. In our opinion, at least two major factors should be recognized as contributing to the shock following the type of muscle trauma employed. As a direct result of the traumatization, there is a pooling and trapping of whole blood in the injured areas. A reduction in circulating blood volume is therefore common to all the experiments reported here. While this blood loss certainly contributes measurably to the induction of the shock condition, it seems improbable that it is sufficient in itself to cause death.

Secondly, there appears to be an important nervous factor concerned in precipitating shock, as shown by the experiments on cordotomy. On the basis of the physiological dissection of the spinal cord, it would seem that two areas, C and D, are important in the response of the animal to shock induced by muscle trauma to the limbs. Area C, which apparently must be bilaterally removed if shock is to be prevented, includes among others the dorsal spinothalamic tracts which, according to clinical and experimental evidence, transmit pain stimuli. These tracts may not be the sole ascending paths for this sensation in mammalia below the primates (8–9), but in the dog they appear to constitute pain conduction paths (10). This does not, of course, rule out the possibility that pain stimuli may also be transmitted up the cord to some extent through a series of short relays as suggested for the cat (8–9). It is perhaps more than coincidence that our best results in preventing shock were obtained when all cord areas other than D, including the grey matter, were removed (fig. 3, III).

The evidence seems to indicate that a flow of nociceptive stimuli from the area of injury courses up some tract or tracts contained in the ventrolateral cord area marked *C* in our diagram. These stimuli apparently act to decrease the resistance of the animal to trauma, and to prevent the rise in blood pressure and effective venous return in the hours following trauma. How this result is brought about we do not know.

[4] These nine animals are not included in the total of 17 complete cord sections listed in figure 3, II, and table 2.

The barrage of noxious impulses is apparently continued over several hours; at least, a prophylactic anesthesia of the leg nerves which is not continued beyond the time of actual traumatization will not adequately protect against shock. On the other hand the greatest flow of such stimuli must be during and immediately following trauma, for when procaine infiltration is delayed for an hour or so after trauma, it can no longer protect against shock.

The fact that complete spinal cord or leg nerve section, which should suffice to remove all afferent nociceptive stimuli, fails to prevent shock is not easily explained. As far as survival after trauma is concerned, destruction of cord area *D* is equivalent to complete spinal section, so that it is reasonable to assume that nerve tracts contained in cord area *D* are concerned in maintaining the resistance of the animal to shock from muscle trauma. Section of the leg nerves would sever any fibers passing to the legs through area *D* and lead to the same end result as complete cord section.

It has been implied that in all series of experiments, a given amount of trauma has produced a similar degree of injury and local fluid loss. The plasma volume loss in animals with the major nerves of the leg blocked by procaine has been shown to be as large as in the controls (table 1). In the relaxed, denervated limb, this assumption may not be valid. That is, the sensitization of the traumatized dog to shock by the destruction of nerve tracts in the *D* area might be attributed simply to a vasodilatation of the blood vessels distal to the cut, produced either directly or indirectly through loss of muscle tone. This dilatation should increase the fluid loss into the limbs during the actual traumatization. The integrity of the *D* area is also important in the hours following the traumatization, for a cord section superimposed upon a procaine infiltration of the leg nerves, made after completion of the trauma, leads to fatal shock. A greater pooling of blood in the relaxed tissues might counteract the beneficial effects of the procaine in blocking the passage of pain stimuli to the higher centers, and render the animal more susceptible to shock.

These experiments appear to afford additional support for the suggestion of several investigators (11–13) that an important nervous factor exists in traumatic shock.

SUMMARY

1. Traumatization of all faces of the thigh muscles by a light rawhide mallet led to fatal shock in 33 (or 89 per cent) of 37 anesthetizd control dogs.

2. A complete section of the spinal cord of 13 animals immediately before trauma did not prevent fatal shock in 12, or 92 per cent of the cases. This failure to prevent death is not related to the trauma involved in the cord section, itself, since 4 more dogs, with cord sections made 3 days previous to the experiment, also died in shock. Thus of a total of 17 traumatized dogs with completely severed cords, but one survived.

3. Likewise cutting the major nerves to the legs prior to trauma, did not prevent death in 11 dogs of a total series of 12.

4. Proper anesthetization of the same nerves with 4 per cent procaine but without preliminary exposure of the nerve, or removal of blood for plasma volume, prevented shock from appearing in 10 of 11 animals.

5. Cordotomy, involving bilateral removal of certain areas, followed by trauma, indicated that afferent nociceptive stimuli from the legs, tending to precipitate shock, are apparently transmitted by fibers contained chiefly in the ventrolateral cord region. These fibers may be the dorsal spinothalamic or pain tracts. Section of the cord except for the ventral or *D* area, followed by trauma, prevented fatal shock in 29 of 34 classes.

6. When the ventral cord or *D* area is sectioned, shock induced by muscle trauma can not be prevented even though the pain tracts may have been removed, or the pain stimuli blocked by procaine anesthesia. Removal of this area is believed to explain the sensitivity to shock of animals with either complete spinal cord or leg nerve section.

7. Two factors apparently contribute to the initiation of shock following the type of muscle trauma employed: *a*, a local loss of whole blood into the traumatized areas, and *b*, a nervous factor.

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THE STATISTICAL ANALYSIS OF THE KNEE-JERK

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Received for publication November 18, 1943

For many years I have been impressed with the variability in height of the knee-jerk taken from normal subjects and by the fact that there are no known standards by which the height may be said to be in the normal range. Yet the height of the knee-jerk expressed as the mean or median has been very useful in establishing norms which are then compared to experimental data taken from the same subject. In this way the knee-jerk has been used as an index to: The activity of the central nervous system (1), localization of injured pathways in brain and cord (2), mental activity (3, 4), relaxation (5), and problems related to muscle tonus (6, 7, 8, 9, 10). These researches were sometimes done on a single subject or at the most the data represent only a few individuals. Thus it would be of value to have information on a large group of subjects. In this report data taken from the knee-jerks of more than twelve hundred students are presented.

METHOD OF STUDY. The subjects were students of medicine and dentistry and represented nearly all members of each class from 1929 to 1940 in the medical group and the classes from 1930 to 1941 in the dental group. There were thirty-six women and these have been treated as a separate group. The knee-jerk apparatus was similar to the one designed by Tuttle (11) and consisted of an iron hammer faced with rubber and a recording device. A leather strap designed to fit the heel of the shoe connected the right foot to a leather thong which fastened to an iron triangle. By this arrangment the amplitude of the knee-jerk was reduced to about one-tenth. A weighted cord and pulley held taut the recording part of the apparatus but allowed for free movement of the foot.

The experiments were carried out in a quiet room to which only a few students at one time were admitted. The subject was blindfolded in all procedures except during the solving of the problem. An instructor closely supervised the taking of records. Various positions of the ligamentum patellae were explored with submaximal stimuli to determine the most sensitive area for eliciting the knee-jerk. The hammer was finally adjusted to deliver a minimal (so-called) stimulus which was described as one that would bring about a visible knee-jerk response and move the foot one or two inches. Fifteen to twenty knee-jerk reactions, at intervals of about five seconds, were recorded as normals. Then, without moving the subject, a second set of reactions, equal in number, was obtained, during which the fall of the hammer was slightly preceded by a sensory stimulus elicited as follows: The hand of the experimenter was supported and remained in contact with the head of the subject and just preceding each fall of the hammer a slight brisk pull of short duration was given the hair above the

ear. Later, without moving the subject, a third record of an equal number of responses was obtained while the hands were clasped, the subject being instructed to clasp just before each stroke of the hammer. Finally, a fourth series of reactions was obtained during the solving of the following problem: "The sum from 1 to any number x is $(\frac{1}{2}x + \frac{1}{2})x$; try this formula for 1 to 10 and compare simple addition."

This gave four records each of which contained 15 to 20 knee-jerk reactions. The student measured each knee-jerk in millimeters and obtained means for the four records, no response being measured as zero and included as such in the total. These mean values, carried to two decimal places, form the data used in this paper.

Analysis of the data. The means, per cent change from normal resulting from each procedure, coefficients of variation, significant ratios, medians and ranges are shown for the medical and dental groups separately and combined in table 1; in addition, the data for the thirty-six women students are given separately.

It is very interesting to find the mean values agreeing so closely as 8.53 and 8.49 mm. for the normal knee-jerk in the medical and dental groups. Due to the large number of subjects these figures have become somewhat fixed and they would change only slightly by the addition of more variates because the probable errors are small being ± 0.13 and ± 0.16 in the medical and dental groups respectively. In the group consisting of women the mean, median and range of the normal knee-jerk are higher than for those for men (table 1). The mean is significantly above that of the men and the "significant ratio" is 3.4. This leads one to wonder if these data can be interpreted as indicating that women are more irritable than men thus confirming the evidence obtained by Williams who reported (11) that the same strength of stimulus (50 grams) applied to the ligamentum patellae induced a greater response in women than in men. In the present study the stimulus was not a fixed one but one selected to give a response that was close to minimal. In sensitive subjects it is more difficult to select a stimulus that is minimal and the variability in the height of the knee-jerk is additive, thus giving a mean value that is always above the recorded minimal of a single response. This is so because we have no way to record the change in muscle tonus induced by a subminimal stimulus and these are recorded as zero. In less sensitive subjects this additive effect is less and the mean value for the normal knee-jerk is lower.

Sensory reinforcement gave an increase in the knee-jerk in all groups but especially so in women. The means for men and for women are very significantly above their normals as shown in the column headed "ratio" of table 1.

There was almost no correlation obtained when the height of the normal knee-jerk was compared to increase induced by sensory reinforcement; the coefficients of correlation being -0.01 and $+0.01$ in medical and dental students respectively (table 2). This lack of correlation with the normal knee-jerk is surprising since one usually thinks that the smaller the initial response the greater chance there would be for any reinforcement to express itself. In other words these data

show that the amount of reinforcement of the knee-jerk induced by pulling the hair is not related to the strength of the blow given the patellar tendon. The knee-jerk was not always increased by sensory stimulation, 52 or 4.3 per cent

TABLE 1

PROCEDURE	MEAN	CHANGE	COEF. VAR.	RATIO	MEDIAN	RANGE
713 students of medicine						
		(%)				
Normal	8.53		55.8		7.99	0.1–28
Sensory	15.09	+76.9	42.6	22.1	14.84	1 –35
Hand clasp	14.48	+69.7	42.3	20.7	14.19	0.5–34
Problem	10.57	+22.6	59.9	6.9	10.21	0.5–37
507 students of dentistry						
Normal	8.49		59.6		7.54	0.6–27
Sensory	14.00	+65.6	46.4	15.4	13.28	1 –41
Hand clasp	13.93	+63.0	42.1	16.2	13.58	1 –37
Problem	9.53	+12.2	60.3	3.4	8.42	0.1–30
1220 students of medicine and dentistry						
Normal	8.51		58.4		7.81	0.1–28
Sensory	14.64	+72.0	44.1	26.1	14.20	1 –41
Hand clasp	14.21	+67.0	42.9	25.3	13.97	0.5–37
Problem	10.14	+19.0	60.5	7.1	9.63	0.5–37
36 women students of medicine						
Normal	12.20		52.8		11.50	2–32
Sensory	17.69	+45.0	29.4	4.0	17.50	5–31
Hand clasp	18.29	+49.2	39.4	4.0	18.50	4–32
Problem	14.62	+19.8	47.6	1.5	14.00	1–31

Data based on the height, in millimeters, of the knee jerk for students of medicine and dentistry are shown separately and the men students are combined. Significant ratio = the difference of two means divided by their standard error of the difference.

$$\text{Coefficient of variation} = \frac{SD \times 100}{\text{Mean}}$$

$$\text{Standard error of difference} = \sqrt{(SE_1)^2 + (SE_2)^2}$$

$$SE = \text{Standard error of mean} = \frac{SD}{\sqrt{N}}$$

$$SD = \text{Standard deviation} = \sqrt{\frac{\Sigma d^2 f}{n}}$$

of the combined medical and dental students showed no change and 46 or 3.8 per cent showed a decrease in the height of the knee-jerk as compared to their normal means. These figures for the women were 2 or 5.5 per cent and 3 or 8.3 per cent for no change and a decrease in the height of the knee-jerk respectively.

Reinforcement of the knee-jerk induced by clasping the hands was significantly above normal and of a similar magnitude as that induced by sensory stimuli; the mean values were less in men than women while the per cent change was more in men than women (table 1).

The coefficient of correlation between the height of the normal knee-jerk and the increase induced by clasping the hands is negative and significant being −0.13 in the medical and −0.19 in the dental groups (table 2). Even greater correlations were obtained (+0.52 and +0.69, table 2) when the increase, above normal, obtained by clasping the hands is compared to the increase obtained by sensory stimulation. This correlation is positive indication that an individual

TABLE 2

The coefficients of correlations (R) are shown for all possible comparisons between the normal knee-jerk, three different types of reinforcement and grades obtained in Physiology

In each square the "R" for students of medicine is given first with its standard error, below this are the "R" and standard error for the students of dentistry. If the "R" is three or more times its standard error the correlation is regarded as significant and marked by an asterisk. Number of students as in table 1. The means (medical above) with their standard errors are shown at the bottom of the table.

$$\text{Standard error of "R"} = \frac{1 - R^2}{\sqrt{n}}$$

Grades					
Normal	−0.01 ±0.038 −0.05 ±0.044				
Sensory reinforcement	+0.05 ±0.037 −0.05 ±0.044	−0.01 ±0.038 +0.01 ±0.044			
Hand clasp reinforcement	+0.03 ±0.037 −0.06 ±0.044	−0.13 ±0.037* −0.19 ±0.043*	+0.52 ±0.027* +0.69 ±0.023*		
Problem reinforcement	+0.02 ±0.038 −0.07 ±0.044	−0.11 ±0.037 −0.16 ±0.043*	+0.29 ±0.034* +0.26 ±0.041*	+0.40 ±0.031* +0.43 ±0.036*	
	Grades	Normal	Sensory reinforcement	Hand clasp reinforcement	Problem reinforcement
	80.1 ±0.306 76.9 ±0.432	8.53 ±0.175 8.49 ±0.224	6.56 ±0.166 5.51 ±0.179	5.95 ±0.169 5.44 ±0.159	2.04 ±0.170 1.04 ±0.189

who gave a high knee-jerk during sensory reinforcement also responded well when the hands were clasped.

Clasping the hands did not always give a higher knee-jerk, 54 or 4.4 per cent of the combined medical and dental students showed no change and 68 or 5.6 per cent showed a decrease from their normal. The figures for the women were zero and 3 or 8.3 per cent for no change and a decrease in the height of the knee-jerk respectively.

When the subject was engaged in mental activity as during the solving of a problem the knee-jerk was increased about 19 per cent above normal in both the men and women (table 1). In the men this change was significantly above

normal as shown by a "significant ratio" of 7.1, but in the women group the number of observations was much smaller and the knee-jerk taken during the solving of a problem was not significantly different from the normal, the "significant ratio" being 1.5 (table 1).

There is a negative correlation between the height of the normal knee-jerk and the increase in height that occurred during mental activity. In case of the medical students this figure is −0.11 or almost three times its standard error while in the dental group it is −0.16 or about four times its standard error (table 2). When the increase in height of the knee-jerk induced by mental activity is compared to the increased height brought about by sensory stimulation and during clasping of the hand the correlations are positive and very significant (table 2). This again emphasizes the fact that an individual who gives a higher knee-jerk during sensory stimulation will also be likely to show reinforcement of a similar magnitude when the knee-jerk is modified by either clasping the hands or by mental activity.

In a considerable number of individuals mental activity did not increase the height of the knee-jerk. In 115 cases or 9.4 per cent of the men the knee-jerk during this period was unchanged and in 368 or 30.2 per cent it was below normal. In 1 or 2.8 per cent of the women the knee-jerk was unchanged and in 8 or 22.2 per cent it was below normal.

Discussion. These experiments covered an eleven year period and during this interval the data were not in any way analyzed for fear that such information might influence the procedure and modify the results. It is very interesting to find that the mean values for the normal knee-jerk and for the three periods of reinforcement are so closely alike in the medical and dental groups. This would seem to indicate that the number of individuals was sufficient or even beyond that required for analysis of data of this kind which represent a measurement of muscle tonus and, therefore, subject to considerable variation as attested by large coefficients of variation.

The interest a student shows in a course is obviously associated with the grade attained. It was thought that an interest in doing well in this experiment might be reflected in the height of the knee-jerk especially when the knee-jerk was reinforced by hand clasping and solving a problem. To test this relationship the student's grade in Physiology has been correlated with each of the four knee-jerk periods. These correlations are small, insignificant and show no trend in either a negative or positive direction (table 2).

A few students gave practically no normal knee-jerk even when the blow delivered to the ligamentum patellae was very strong; five of the medical students gave mean values of less than 1.0 mm. and in two of these the knee-jerk was 0.1 mm. in height. In the dental students the number below 1.0 mm. was four and in three of these the mean was 0.1 mm. Such low mean values are made possible because the record shows a vacant space when the knee-jerk was zero and these spaces counted in the number of responses.

Reinforcement was one of the problems dealt with in the first knee-jerk studies (12, 13) and in hundreds of papers since then. Therefore, no one questions its occurrence but what is it related to and what is its magnitude? One

factor that it is obviously compared to is the height of the normal knee-jerks taken just previous to the reinforcement. Yet as we have seen this relationship is not fixed and when the normal height is correlated with the height induced by sensory stimuli (pulling the hair), overflow of impulses (clasp of the hands), or mental activity (solving a problem) the resulting figures are not impressive, being −0.01, +0.01, −0.13, −0.19, −0.11, and −0.16 for the "R" in each of the six possible comparisons given in table 2. Neither is the effect of reinforcement of the knee-jerk always predictable in direction nor in magnitude of change from normal. For example, 483 or 39.6 per cent of the 1220 men subjects failed to show any reinforcement of the knee-jerk when solving a problem and 122 or 10.0 per cent not only failed to show reinforcement as a result of clasping the hands but many of them gave knee-jerks that were less than normal. The magnitude of the change brought about by attempts to reinforce the knee-jerk is likewise unpredictable and individual subjects varied widely. The per cent change from the normal mean showed a range of −96 to +551 per cent, −67 to +568 per cent and −100 to +687 per cent during periods of reinforcement due to sensory stimuli, hand clasping and mental activity respectively. One must bear in mind that these were untrained subjects and that no attempt was made to correct any of the results by a subsequent experiment. In some cases failure to obtain a higher knee-jerk may have been due to faulty timing of the reinforcement as others have shown (13). This criticism is not applicable to the results obtained during a period of mental activity nor is it always likely to be valid for the other two periods of reinforcement obtained in these subjects because the application of reinforcement was timed with reasonable care and also because the analyses are based on mean values representing 15 to 20 reactions for each period.

SUMMARY

Knee-jerk records were obtained in 1256 students. Changes induced by sensory stimuli, clasping the hands and mental activity were studied in each subject.

The mean height of the normal knee-jerk varied widely and the coefficient of variation for the normal was often larger than it was during periods of reinforcement. The changes induced by reinforcement were not predictable in either direction or magnitude. Correlations between height of the normal knee-jerk and height obtained by reinforcement are very low and the coefficient of correlation ranged from −0.19 to +0.01.

Mental capacity as represented by grades in physiology is not related to the height of the normal knee-jerk or to the changes induced by reinforcements.

Correlations between the height of the knee-jerk reinforced by sensory stimuli, by clasping the hands and by mental activity are very high and the coefficient of correlation ranged from +0.26 to +0.69.

The knee-jerk responses of medical and dental men students were quite alike. In women hypersensitivity of this reflex was evident.

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THE RELATION OF URIC ACID EXCRETION TO BLOOD LACTIC ACID IN MAN

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Received for publication November 22, 1943

An increase in blood lactic acid has been observed during hippuric acid synthesis from ingested sodium benzoate in man (1). The changes in blood lactate paralleled the trend of the uric acid retention which occurs after ingestion of sodium benzoate. As uric acid retention also occurs after ingestion of lactate (2) it was decided to study changes in blood lactic acid which were expected to occur following ingestion of lactate and to correlate these with the uric acid excretion.

METHODS. The subjects of the study were male schizophrenic patients with no clinical evidence of physical disease. All subjects were studied in a fasting state and were reclining in bed during the period of observation. Five urine samples were collected in approximately one-hour periods, the first timed sample serving for control comparison. Approximately 10 minutes before the end of the first (control) period, 2.5 milliequivalents of lactic acid[1] per kilogram body weight, partially neutralized with 0.8 equivalent of sodium hydroxide for each equivalent of lactic acid, was ingested and followed by a glass of water. One drop of oil of peppermint was added to each dose as a corrigent of taste. Under these conditions a slightly higher amount of lactic acid was found in those samples which were collected at least 10 minutes after ingestion of the lactic acid. It was therefore deduced that absorption of lactic acid began within a few minutes following ingestion.

Blood samples were collected from a cubital vein, with a minimum amount of stasis, preceding the ingestion of lactate and at the half-times of several periods following the ingestion of lactate. One glass of water was ingested before each voiding (after collection of blood samples) to facilitate urine sampling. Uric acid in urine and serum was determined by the method of Folin (3), pyruvic acid in trichloracetic blood filtrates was estimated as its di-nitrophenyl-hydrazone by the modification of Bueding and Wortis (4), and lactic acid in the same filtrates by the method of Barker and Summerson (5). The latter method was also successfully applied to the estimation of lactic acid in diluted urine.

RESULTS. The blood lactic acid values were considerably higher during the first and second hours following the lactate ingestion when compared to the pre-test levels. The highest values ranged from 12.2 to 18.5 mgm. lactic acid per 100 ml. blood. The average increase in 6 subjects in whom samples were obtained during the first or second period following ingestion of lactate was 8.4 mgm. per 100 ml. of blood. A concomitant increase in the pyruvic acid level accompanied the increase in lactic acid.

The uric acid excretion decreased in all subjects who ingested 2.5 milli-equiva-

[1] "Reagent Mallinckrodt."

lents of lactic acid per kilogram body weight. The lowest rate of uric acid excretion occurred during the first or second period following ingestion of lactate and was approximately one-half that of the pre-test period. No decrease in uric acid excretion occurred in two subjects who had ingested only 1.6 milliequivalents of lactic acid per kilogram body weight.

The decrease in uric acid excretion was not proportional to the increase in blood lactic acid. However, a significant correlation was found between the absolute blood lactic acid level and the uric acid excretion rate in samples collected for four hours following the ingestion of lactate (cf. fig. 1). The correlation coefficient between these two values was -0.82 ± 0.051.

The urinary excretion of lactic acid increased markedly following ingestion of lactate. The lactic acid excretion was somewhat correlated to the blood lactic

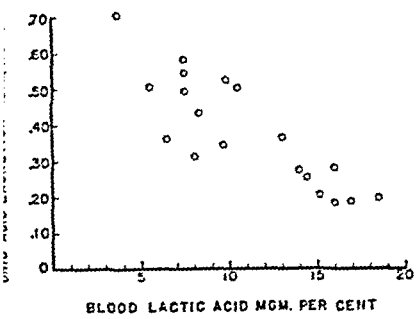

Fig. 1

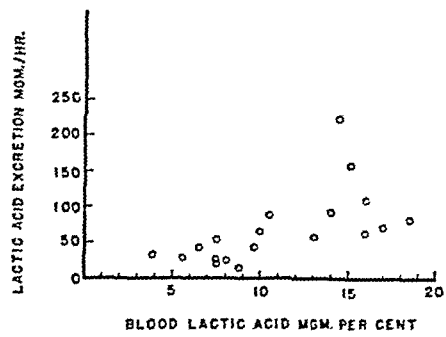

Fig. 2

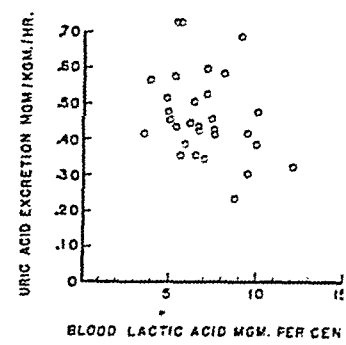

Fig. 3

Fig. 1. Scatter-diagram showing relationship between uric acid excretion rate in milligrams per kilogram body weight per hour (ordinate) and blood lactic acid in milligrams per 100 ml. blood after ingestion of lactic acid. Correlation coefficient = -0.82 ± 0.051 (7 subjects).

Fig. 2. Scatter-diagram showing relationship between lactic acid excretion in milligrams per hour (ordinate) and blood lactic acid in milligrams per 100 ml. blood after ingestion of lactic acid (7 subjects).

Fig. 3. Scatter-diagram showing relationship between uric acid excretion rate in milligrams per kilogram body weight per hour (ordinate) and blood lactic acid in milligrams per 100 ml. blood in 25 fasting and resting subjects.

acid levels (cf. fig. 2), although it remained considerably above the pre-experimental rate even after the blood lactic acid returned to and fell below the initial level (subject J. K. in table 1).

Comment. The good correlation of the uric acid excretion with the blood lactic acid level seems to indicate that the retention of uric acid following ingestion of lactate is effected through a change in the blood lactic acid level. The lack of linearity of the correlation in the individual subjects (cf. table 1) suggests that additional factors enter into the mechanism.

When the changes in the blood lactic acid and in the uric acid retention following lactic acid ingestion were compared with those which occur during hippuric acid synthesis, it was noticed that the increase in blood lactic acid was significantly smaller following sodium benzoate ingestion (average 1.5 mgm. per cent

in 24 subjects) even though the uric acid retention was more pronounced than it was following lactic acid ingestion. There was no significant correlation between the blood lactic acid and the uric acid excretion following sodium benzoate ingestion (correlation coefficient = -0.29 ± 0.100) as contrasted with the good correlation following lactic acid ingestion.

Benzoic acid rated on an equi-molecular basis had a more powerful uric acid retaining effect than had lactic acid. The lowest uric acid excretion rate following ingestion of 2.5 milli-equivalents of lactic acid per kilogram body weight averaged 0.476 of the pre-experimental excretion rate in 7 subjects, while the ingestion of 0.55 milli-mol of sodium benzoate per kilogram body weight lowered the uric acid excretion to 0.238 of the pre-experimental excretion (24 subjects).

TABLE 1

Changes in the blood levels of lactic and pyruvic acids, and in the urinary excretion of uric and lactic acids following ingestion of lactic acid in two representative subjects

URINE SAMPLES				BLOOD SAMPLES			
Time	Volume	Lactic acid	Uric acid	Time	Lactic acid	Pyruvic acid	Serum uric acid
Subject A. D., age 39, weight 54 kgm., 0.135 equivalent of lactic acid ingested at 8:55 a.m.							
	ml.	*mgm./hour*	*mgm./hour*		*mgm./100 ml.*	*mgm./100 ml.*	*mgm./100 ml.*
8.07–9.07	150	3.9	21.0	8.28	5.8	0.63	4.1
10.04	104	86.4	11.0	9.33	18.5	1.07	
11.06	67	63.6	10.0	10.35	16.0	1.21	
12.08	241	44.8	18.9	11.38	9.7	0.84	
1.04	272	27.1	17.2	12.39	8.1	0.77	
Subject J. K., age 20, weight 64 kgm., 0.161 equivalent of lactic acid ingested at 8:50 a.m.							
8.05–9.01	244	11.8	31.9	8.23	8.0	0.83	
10.00	120	160.0	13.5	9.27	15.2	1.21	3.9
11.00	66	113.5	18.6	10.31	16.0	1.23	
12.04	124	86.8	32.4	11.30	10.5	0.86	
1.00	142	43.4	23.7	12.34	6.5	0.74	

On the basis of these results it is estimated that benzoic acid is approximately 10 times more effective in retaining uric acid than is lactic acid on an equi-molecular basis.

If the retention of uric acid following sodium benzoate ingestion were to be mediated through changes in the blood lactic acid level the latter would be expected to increase to values much higher than actually observed under the experimental conditions. The changes in the blood lactic acid level following ingestion of benzoate are apparently only tangential to the main pathway of the causative mechanism of the uric acid retention as contrasted with the conditions following lactate ingestion, in which the blood lactate changes form a quantitative link of the mechanism.

Uric acid excretion correlates with the blood lactic acid level also under condi-

tions in which no outside intervention is imposed upon the organism. Thus, a correlation was found between the two values in samples obtained from 25 resting and fasting subjects who were not undergoing any experimental procedures. The correlation coefficient -0.42 ± 0.101 is relatively low (cf. fig. 3).

On the basis of these three independent tests of correlation it may be concluded that uric acid excretion is linked with the level of blood lactic acid. The observations after lactic acid ingestion imply that the blood lactic acid level may have controlling influence on the excretion of uric acid by the kidney.

SUMMARY

The relation of the blood levels of lactate and pyruvate to the excretion of uric acid by the kidney following ingestion of lactic acid was investigated and the results compared with the blood-lactate increase observed after the ingestion of sodium benzoate.

The uric acid excretion was correlated to the blood lactic acid level for four hours following ingestion of lactic acid, the correlation coefficient being -0.82 ± 0.051.

Following lactate ingestion, the blood lactic acid increased to values considerably higher than found after ingestion of benzoate, although the reverse was found for the retention of uric acid which was higher after benzoate ingestion.

A moderate correlation of uric acid excretion with the blood lactate level was observed in 25 resting and fasting subjects.

The correlation between the blood lactic acid level and the uric acid excretion is considered evidence of a controlling influence of some phase of lactic acid metabolism on uric acid excretion.

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A POSSIBLE MODE OF ACTION OF PEDICLE JEJUNAL GRAFTS ON GASTRIC SECRETION AS INDICATED BY CHANGES IN pH OF THE SURFACE OF THE MUCOSA OF THE STOMACH[1]

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Received for publication November 27, 1943

While the conventional analysis of gastric juice aspirated from the stomach may ideally give evidence as to the secretory activity of the stomach as a whole, it fails to indicate the acidity of any given spot on the surface of the mucosa and does not permit a comparison of the reactions of different regions of the stomach to a given stimulus. In studying the effects of pedicle jejunal grafts in the wall of the stomach on gastric secretion in animals we have therefore employed in addition the technic of direct measurement of the pH of the surface of the gastric mucosa by inserting the electrodes of the Beckman pH meter through a gastrotomy.

METHOD. Ten normal mongrel dogs of both sexes were used in these experiments which were carried out under nembutal intravenously (0.2 grain per pound of body weight) or inhalation ether anesthesia. No differences in response could be traced to the anesthetic agent employed. After making a gastrotomy on the anterior surface of the stomach about midway between pylorus and cardia the electrodes of the pH meter were inserted and applied with firm steady pressure to the desired point on the surface of the mucosa. When contact is established there is an immediate deflection of the indicating needle of the meter which remains steady as long as contact is maintained—up to 10 minutes in instances in which this point was specifically investigated. After the animals had had pedicle grafts implanted, usually about midway between cardia and pylorus on the anterior wall of the stomach, the gastrotomy was made just cephalad to the implant.

In this manner seven different regions of the anterior of the stomach were examined, the pylorus (P), the anterior (A.A.) and posterior (P.A.) surfaces of the antrum, the lesser (L.C.) and greater (G.C.) curvatures at about their mid points, the posterior surface of the fundus (F) and the cardia (C.). After making the first series of observations the animals were given 1 mgm. of histamine phosphate subcutaneously and further observations carried out 10 and 20 minutes later, at which time previous studies had indicated that the secretion of acid was at its height. All the data so obtained are recorded in table 1. In order to give a more or less composite picture the logarithms of the averages of the antilogarithms of the individual pH determinations are included.

RESULTS. No significant differences in the fasting examinations before and after the implantation of the jejunal grafts are demonstrable. In 38 instances

[1] This study was carried out under a grant from the John and Mary R. Markle Foundation.

the pH fell slightly while in 32 it rose, but the total change was of no significance and no pattern was evident.

Ten minutes after the administration of histamine, however, the picture was strikingly altered. In 61 instances the pH of the surface of the mucosa of the

TABLE 1

pH of the surface of the mucosa of 7 regions of the stomach before and after jejunal implant

ANIMAL NO		FASTING								10 MIN. AFTER HISTAMINE								20 MIN. AFTER HISTAMINE							
		P	AA	PA	LC	GC	F	C	Log. of average of antilogs	P	AA	PA	LC	GC	F	C	Log. of average of antilogs	P	AA	PA	LC	GC	F	C	Log. of average of antilogs
1	B1	4.9	3.9	4.6	6.6	3.1	2.9	2.9	4.3	2.2	4.2	2.0	3.4	1.8	1.8	1.3	2.5	3.6	1.0	1.5	1.5	1.3	1.5	1.1	1.7
	A1	5.9	6.7	6.5	6.2	6.3	3.6	2.8	5.7	5.8	6.8	6.6	7.1	7.1	6.8	2.5	6.3	6.4	7.6	7.4	7.5	6.8	6.9	1.9	6.6
2	B1	6.2	6.0	5.8	3.3	4.4	6.8	6.8	5.8	2.5	5.9	5.3	1.3	2.7	3.2	2.0	3.6	1.1	4.0	3.6	1.2	1.6	1.3	1.2	2.2
	A1	6.8	7.3	6.5	5.4	2.4	2.0	1.7	5.1	6.9	6.8	6.7	7.1	6.8	5.7	2.6	6.3	6.7	7.1	6.0	7.0	6.8	5.0	6.5	6.5
3	B1	5.4	6.2	5.1	4.8	6.9	6.7	2.9	5.6	3.7	2.8	2.7	1.8	3.4	5.8	3.9	3.8	4.2	3.4	2.3	1.6	1.7	1.9	2.5	2.6
	A1	6.4	6.3	6.5	7.1	6.8	7.3	6.8	6.8	2.0	4.2	6.9	7.1	5.2	6.3	6.2	5.7	4.3	4.3	5.0	1.9	3.0	6.4	5.1	4.5
4	B1	3.5	6.2	4.6	6.1	6.9	5.6	5.6	5.6	1.6	4.5	4.8	1.2	4.7	4.7	1.1	3.5	1.2	4.6	4.5	1.2	1.0	1.0	1.1	2.4
	A1	2.9	4.3	5.9	1.9	3.1	2.8	2.0	3.2	6.2	6.5	6.8	6.7	5.5	5.2	2.4	5.8	6.1	6.8	5.6	6.9	6.9	5.4	5.1	6.2
5	B1	3.2	2.8	1.4	3.1	4.3	4.0	4.0	3.3	1.6	1.1	1.3	1.5	1.2	2.0	1.6	1.5	1.5	0.7	1.5	1.5	1.2	1.9	2.0	1.4
	A1	3.9	3.9	3.6	5.6	2.5	2.3	1.8	3.5	6.1	6.2	5.6	6.8	7.1	7.4	6.2	6.5	6.4	6.5	5.7	6.0	5.4	5.1	6.5	6.0
6	B1	7.2	7.2	6.3	7.1	6.8	6.4	3.5	6.5	1.2	1.3	1.3	7.1	1.1	1.1	1.3	2.7	0.9	0.9	1.1	0.8	1.4	0.9	1.0	1.0
	A1	2.2	6.5	3.5	2.8	4.6	3.8	2.1	3.9	4.7	6.4	6.7	6.5	5.3	5.3	2.4	5.5	5.5	6.8	6.3	5.5	6.0	4.0	3.4	5.5
7	B1	6.7	6.6	6.8	6.5	4.4	4.0	3.4	5.7	7.1	2.3	2.4	6.6	2.2	3.0	2.1	4.2	2.0	2.1	1.7	1.7	2.5	2.6	2.3	2.1
	A1	2.9	6.9	7.9	6.7	6.0	7.0	6.6	6.5	6.2	7.1	7.1	6.7	6.5	7.4	5.1	6.6	6.9	7.1	7.2	7.0	7.0	7.4	6.3	7.0
8	B1	2.6	2.5	3.2	2.3	4.5	4.3	1.6	3.1	3.4	2.7	3.7	4.2	1.4	1.3	1.4	2.7	2.7	1.4	2.1	1.8	1.3	1.3	1.1	2.1
	A1	2.0	2.3	2.0	5.3	2.7	1.5	1.7	2.7	1.7	1.5	4.3	4.1	5.4	5.6	2.0	3.8	6.8	2.4	2.1	6.7	2.1	6.0	1.5	4.5
9	B1	6.5	6.2	6.7	6.5	6.8	6.4	6.7	6.6	4.1	5.8	4.8	4.0	3.5	2.8	4.7	4.4	4.4	5.5	2.2	3.7	1.9	2.5	3.6	3.6
	A1	6.3	5.2	6.3	5.9	5.3	6.1	6.2	5.9	6.6	6.5	5.9	5.9	7.0	6.2	6.0	6.3	4.8	6.1	5.3	5.9	5.4	6.2	5.2	5.6
10	B1	7.2	3.9	4.5	4.5	3.0	3.5	3.5	4.5	7.2	1.8	6.2	4.8	3.5	2.3	2.6	4.5	7.0	1.6	3.0	0.8	1.3	1.5	1.3	2.9
	A1	1.5	5.6	2.0	5.6	7.0	1.6	1.3	4.1	2.0	6.7	3.2	6.8	6.7	3.2	2.2	4.9	1.8	7.1	5.1	7.0	7.0	6.2	2.9	5.7
Logs of averages of antilogs	B1	5.6	5.0	5.2	5.4	5.4	5.3	5.3	5.2	4.0	3.6	3.6	4.1	2.7	3.0	2.4	3.3	3.3	2.9	2.5	1.7	1.6	1.7	1.8	2.3
	A1	4.5	5.7	5.5	5.5	5.0	4.4	3.9	4.9	5.2	6.1	6.1	6.6	6.3	6.1	4.1	5.9	5.8	6.4	5.8	6.3	5.9	6.0	4.9	6.1

B1 = before jejunal implant.
A1 = after jejunal implant.

same region of the stomach was higher in the grafted animal than before the graft had been implanted, while in 9 it was slightly lower. The logarithm of the average antilogarithm representing the pH of the seven regions of the stomach tested was considerably higher in the grafted animals as compared to that in the normal, and of perhaps even greater importance was significantly higher than

that of the grafted animals themselves during the fasting examination. Moreover, the greatest rises in pH were found in those regions of the stomach in which the secretion of acid is usually most active.

The picture was further developed 20 minutes after histamine, 68 of the 70 tests in the 10 grafted animals showing a rise in pH. While the pH in the normal animals tended to reach still lower levels, that in the experimental series moved in the opposite direction. The secretion of the graft studied both by measurements of the pH of the graft after implantation and of washings of isolated jejunal loops has been found to be from 5.5 to 6.9 and to contain no appreciable titratable buffer (1).

SUMMARY

A survey of these data supports the idea that a pedicle jejunal graft implanted in the wall of the stomach exerts a direct effect upon the acid secretory mechanism since in the pattern of the alterations the greatest changes tend to occur in that part of the stomach in which the acid secreting glands are most plentiful. Further, since these changes are consistent and much more marked after histamine, it would appear in this instance at least that the graft exerts its effects upon some phase of gastric secretion in which histamine plays an important rôle.

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HISTAMINE IN HUMAN GASTRIC MUCOSA[1]

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Received for publication November 27, 1943

It was first noted by Edkins (1906) that aqueous extracts of the pyloric mucosa stimulated gastric secretion when injected intravenously into cats. In Edkins' experiments extracts of the fundic mucosa did not stimulate gastric secretion. Edkins and Tweedy (1909) named the active principle in these extracts gastrin. Popielski (1920) demonstrated that histamine caused secretion of gastric juice and associated this substance with vasodilatin which he had found similar to Edkins' gastrin and which he found could be extracted from a wide variety of tissues including both fundic and antral mucosa. Sacks, Ivy, Burgess and Vandolah (1932) isolated histamine in crystalline form from hog pyloric mucosa. Gavin, McHenry and Wilson (1933) studied the histamine content of gastric mucosa and gastric muscle from dogs. They found that the mucosa had a much higher content of histamine than the muscle and that the fundic mucosa contained more histamine than the antral mucosa. We have made a study of the histamine activity extractable from the gastric mucosa of various regions of the stomach in man.

METHODS. The mucosa was obtained from specimens removed surgically during the course of gastric resection. Specimens of mucosa were obtained from ten patients. Six of the patients had a benign ulcer, either gastric or duodenal; four of the patients had gastric carcinoma. Three of the patients had achlorhydria as indicated by no free hydrochloric acid in the aspirated gastric contents after the subcutaneous injection of 0.5 mgm. histamine (table 1).

Immediately after its removal, the stomach was laid open along the greater curvature and representative regions of the antral and fundic mucosa were separated by sharp dissection. After being trimmed to an approximate weight of 6 grams, each specimen was weighed accurately, then placed in a porcelain mortar and ground with clean sand. Forty to 50 cc. of a 10 per cent solution of trichloracetic acid were gradually added, the grinding being continued until a homogeneous mixture was obtained. The mixture was then filtered through paper using a Buchner funnel and mild suction. The precipitate was washed repeatedly with 5 per cent trichloracetic acid. When the washing was completed, the entire filtrate was transferred to a graduate cylinder and made up to a convenient volume, usually between 60 and 80 cc., with 5 per cent trichloracetic acid. Because histamine is stable in acid solution, the highly acid filtrate could be stored at this stage for long periods in the ice box without deterioration of its

[1] The researches presented here were supported by grants from the Graduate School of the University of Minnesota and by a grant for technical assistance by the Work Projects Administration, Official Project no. 165-1-71-124, Subproject no. 399.

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histamine activity. When the extraction and histamine estimation were to be completed, the filtrate was divided into equal parts and these were treated as duplicate samples.

In a few preliminary observations the trichloracetic acid was removed from the filtrate by shaking with ether in a separating funnel, discarding the ether and repeating the procedure until the filtrate was neutral to Congo paper. The filtrate thus freed of the trichloracetic acid was evaporated to dryness. The dried residue was dissolved in about 10 cc. of physiologic saline solution, neutralized, and its ability to induce gastric secretion tested by injection into dogs

TABLE 1

The histamine activity of fundic and antral gastric mucosa in man

NUMBER, AGE AND SEX	CLINICAL DIAGNOSIS	PATHOLOGIC DIAGNOSIS OF RESECTED SPECIMEN	PREOPERATIVE MAXIMAL FREE ACID SECRETED TO HISTAMINE	HISTAMINE ACTIVITY (MILLIGRAMS PER KGM. MUCOSA)	
				Fundic	Antral
			degrees		
1. 57 F	Chronic duodenal ulcer with retention	Mild chronic gastritis	102	3.5	3.4
2. 38 M	Chronic duodenal ulcer with retention	Mild chronic gastritis	64	20.6	12.5
3. 29 M	Duodenal ulcer, partial retention	Mild chronic gastritis	74	5.6	4.4
4. 53 M	Carcinoma of cardia	Benign gastric ulcer present	0	10.2	
5. 31 M	Gastric ulcer with retention	Normal tissue	76	6.7	
6. 51 M	Gastric ulcer with retention	Normal tissue	86		3.0
7. 60 F	Carcinoma of antrum	Adenocarcinoma present	0	6.6	
8. 56 M	Carcinoma of antrum	Adenocarcinoma present	0	6.5	6.4 (tumor)
9. 70 M	Carcinoma of antrum and retention	Scirrhous carcinoma present	32	24.1	
10. 63 M	Carcinoma of antrum	Mucinous carcinoma present	72	7.8	

with Heidenhain types of gastric pouches. All samples treated in this manner evoked a definite secretion of gastric juice.

In the ten cases reported in this paper, the trichloracetic acid filtrates were further purified and, in addition to injection into dogs with Heidenhain types of pouches, a quantitative assay of the histamine activity of the extracts was made. The procedures used were those described for the quantitative estimation of histamine activity in blood (Code, 1937). Ten cubic centimeters of concentrated hydrochloric acid was added to each of the duplicate samples of the trichloracetic acid filtrate and these were boiled for one and a half hours (Barsoum and Gad-

dum, 1935). After boiling in the presence of hydrochloric acid, the samples were dried three times *in vacuo* in the presence of alcohol. Either the dried residue was taken up in water, filtered, neutralized, made up to a definite volume and then assayed, or an additional extraction with absolute alcohol was performed as a further means of purifying the extract. When extracting with alcohol, the dried residue was treated three times with 10 to 15 cc. of alcohol, with filtration between additions. Fifteen minutes or more was allowed between additions of the alcohol to obtain as complete histamine extraction as possible. The alcoholic filtrate was dried *in vacuo*, the residue taken up in water, filtered, neutralized, and then made up to volume for assay.

The quantitative estimation of the histamine activity contained in the extracts was made on a portion of guinea-pig ileum suspended in Tyrode's solution. As a routine, atropine was added to the stock of Tyrode's solution to minimize possible contraction of the ileum to choline compounds. The results of this assay were expressed as milligrams histamine (base) activity per kilogram gastric mucosa.

When the estimation had been completed on the guinea-pig ileum, the gastric secretory action of the extracts was tested by subcutaneous injection in dogs with Heidenhain types of gastric pouches. Prior to receiving the injection the dogs had been fasted for thirty hours and the rate of fasting secretion from the pouches determined by collection for a number of half-hour periods. The free and total acidity of the samples of gastric juice was determined by titration with one-tenth normal sodium hydroxide using Töpfer's reagent and phenolphthalein as indicators. The free hydrochloric acid values were used in calculating the milligrams of hydrochloric acid output from the pouch.

Results. The histamine activity extractable from the human gastric mucosa was equivalent to from 24.1 to 3.0 mgm. histamine per kilogram mucosa (table 1). In nine cases extracts were prepared from fundic mucosa. The histamine activity of this fundic mucosa was equivalent to from 3.5 to 24.1 mgm. histamine per kilogram mucosa, with an average value of 10.2 mgm. histamine per kilogram mucosa. Extracts of antral mucosa were made in four cases. The histamine activity of these extracts ranged from 3.0 to 12.5 mgm. histamine per kilogram mucosa, with an average value of 5.8 mgm. histamine per kilogram mucosa.

In three of the ten cases enough of the stomach was removed at operation to allow the preparation of extracts from both fundic and antral mucosa. In one instance the antral mucosa contained much less histamine activity than the fundic mucosa (case 2, table 1). In the other two cases, although lower values occurred with the antral mucosa, the differences were too small to be considered significant (cases 1 and 3, table 1).

No significant difference was found between the concentration of histamine activity in mucosa from stomachs which secreted free hydrochloric acid and that in mucosa from stomachs which did not secrete free hydrochloric acid in the preoperative tests. Likewise, no significant difference was noted between cases in which ulcer of the stomach or duodenum was present and those in which carcinoma of the stomach was present (table 1). In the one case in which it was

studied, the histamine activity of the cancerous tissue was 6.4 mgm. per kilogram and thus within the range found for the normal tissue.

The secretagogic activity of all of the extracts was tested in dogs with Heidenhain pouches. In every instance the subcutaneous injection of the extracts produced a secretion of acid gastric juice in excess of that observed during the basal or fasting period. The magnitude of the response was small, the pouches generally secreting 20 to 50 mgm. of free hydrochloric acid with a range of 9.2 to 138 mgm. free hydrochloric acid. The assay on the guinea-pig ileum showed that the quantity of histamine activity contained in the injected extracts ranged from 15 to 120 micrograms. Such small amounts of histamine, when given to dogs with Heidenhain pouches, generally produce secretory responses of about the same order as those obtained with the extracts.

COMMENT. The results of these experiments indicate that human gastric mucosa contains histamine. However, until histamine is actually isolated in crystalline form from human gastric mucosa, a final conclusion cannot be drawn. Throughout this report the precaution has been taken to refer to the activity assayed as "histamine activity," although the evidence is in favor of the view that the activity was due to histamine.

The concentration of histamine activity found in human gastric mucosa is considerably less than that found in canine gastric mucosa by Gavin, McHenry and Wilson. The consistent difference noted by these authors between the pyloric and fundic mucosa in the dog was not observed for man. In four fasting dogs they found an average histamine content of 69 mgm. per kilogram fundic mucosa and 39 mgm. per kilogram pyloric mucosa, whereas in this study the average histamine content of the nine samples of fundic mucosa was 10.2 mgm. histamine and the average value for the four extracts of antral mucosa was 5.8 mgm. histamine per kilogram. The dog generally secretes a more highly acid gastric juice than that encountered in man, and the results raise the question whether or not this may be correlated with a higher histamine content of the gastric mucosa of the dog. Rather opposed to this possibility is the fact that in the series of human cases studied there was no correlation between concentration of acid in the juice from the intact stomach and the histamine content of the gastric mucosa. It should be pointed out that all of the cases studied had pathologic lesions in the stomach. The three cases of the series with achlorhydria for example had either gastric carcinoma or gastric ulcer and some degree of gastritis associated with the achlorhydria. Such cases are not suitable for tests designed to show the presence or absence of a correlation between the amount of acid a mucosa may secrete and the amount of histamine it may contain. The histamine content of the gastric mucosa of patients who have achlorhydria without associated carcinoma or ulcer might yield interesting results.

The quantity of histamine activity found in the gastric mucosa of man, although less than that of the dog, was nevertheless sufficient to evoke gastric secretion when injected into a dog. The extracts injected into the dog usually represented about 5 grams of mucosa. Thus, in 5 grams of mucosa enough activity was present so that if it were released *in vivo* it could act as a humoral gastric

secretory agent. Also, if released in the mucosa *in vivo* it would most likely evoke a marked secretion from gastric glands in the vicinity. In human gastric mucosa, therefore, three is present sufficient histamine activity to allow local and humoral stimulation of gastric secretion.

During this study no systematic search was made for activity in the extracts other than histamine. It may be stated, however, that throughout the assays on the guinea-pig's ileum and the tests on the dog, no activity was encountered which could not be directly attributable to the presence of histamine.

SUMMARY

The histamine activity extractable from human gastric mucosa was studied in ten cases. In nine of the cases extracts were made of the fundic mucosa and were found to contain activity equivalent to 3.5 to 24.1 mgm. histamine per kilogram fundic mucosa, with an average value of 10.2 mgm. histamine per kilogram mucosa. In four cases, extracts were made of antral mucosa. The histamine activity of these extracts ranged from 3.0 to 12.5 mgm. histamine per kilogram mucosa, with an average value of 5.8 mgm. histamine per kilogram mucosa. In three cases the histamine activity of the fundic mucosa was compared with that of the antral mucosa. The antral mucosa contained much less activity than the fundic mucosa in one case, but in the other two the concentration was approximately the same. Upon subcutaneous injection into dogs with Heidenhain pouches all of the extracts produced a prompt secretion of acid gastric juice. The possible significance and some of the limitations of these results are discussed.

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THE EFFECT OF VITAMIN B COMPLEX DEFICIENCY ON THE WATER CONTENT OF THE BODY AND VARIOUS ORGANS OF THE ALBINO RAT[1, 2]

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Received for publication December 4, 1943

On the basis of various clinical observations it is frequently assumed that edema may result from vitamin B_1 deficiency. At least one-third of all cases of beri-beri, which is generally regarded as a vitamin B_1 deficiency disease, are said to be associated with edema. Diuresis and subsidence of edema have been observed following the administration of thiamin in patients with a history of malnutrition and with signs and symptoms characteristic of deficiency in this vitamin (1, 2). More recent investigations, however, indicate that thiamin deficiency does not lead to edema formation (3). In a carefully controlled clinical study, female subjects were maintained for eighty-eight days on a diet supplying not more than 0.15 mgm. thiamin daily, which is approximately one-seventh of the amount regarded as the minimal daily requirement. The diet was adequate in vitamin A, ascorbic acid, minerals, riboflavin and nicotinic acid. Symptoms and signs of vitamin B_1 deficiency, such as depressed mental states, nausea, vomiting and loss of weight, developed, but there was no edema in any of the subjects.

While it would appear from these studies that the edema which is frequently found in beri-beri can not be attributed to a deficiency of thiamin, there remains the possibility that it may be caused by an insufficiency in the intake of other fractions of the B complex. Information on this subject should be of interest not only because of any bearing it may have on nutritional edema, but also for the contribution it may offer toward the elucidation of the mechanism which normally maintains a constancy in the water content of the tissues. The present investigation in which the albino rat was used as the experimental animal was undertaken to determine whether any particular fraction of the vitamin B complex is required for the maintenance of water balance. It was necessary to ascertain first whether any alteration in the water content of the various organs and of the entire body occurs when the animals are fed a ration containing none of the B complex while supplying all other essential nutrients in proper proportions.

METHOD. Thirty-two pairs of litter mates approximately 60 days old were selected from the colony and divided into experimental and control groups. The experimental animals were fed a vitamin B complex free ration[3] for four weeks and the controls the same ration mixed with dry brewer's yeast in the ratio of 10

[1] Preliminary report, Federation Proc. 2: 18, 1943.

[2] The expense of this investigation was defrayed in part by a grant from the University Center of Georgia Research Fund.

[3] Obtained from the Research Laboratories of the S.M.A. Corporation.

to 1. All animals were given daily 7 drops of a 6 to 1 mixture of cod liver oil and linoleic acid. A four week period of feeding was decided upon after preliminary experiments in which it was found that a number of animals did not survive on the B-free ration beyond six weeks. The deficiency syndrome was quite pronounced at the end of four weeks. Anorexia was prominent and there was an appreciable loss of weight. The animals were apathetic when left alone but irritable when disturbed.

As the food intake is greatly reduced on a B-free ration because of the loss of appetite, it was necessary to run two sets of experiments to compare the effects of a diminished food intake with those resulting from the absence of the vitamin complex. In one series the experimental and control animals were allowed to eat *ad libitum* while in the other the caloric intake of litter mates was equalized. The caloric value of the two rations was ascertained by combustion of aliquots in a bomb calorimeter. In the balanced experiments practically the same amount of food was consumed by the experimental and control groups since the caloric value of the two rations was approximately equal.

At the conclusion of the experiment the animals were fasted for 24 hours with free access to water and then decapitated. The blood was collected in weighing bottles and the brain, liver, kidneys, gastrocnemius and a portion of the skin of the back in the region of the shoulders were taken for determination of the water content. Immediately prior to decapitation the hair was clipped off the portion of the skin marked for excision. The remainder of the body was ground first in a sausage grinder and then in a corn mill. This procedure has been found to yield a homogeneous mass (4). An aliquot of 5 to 10 grams was taken from the ground mass for analysis of the water content. All these manipulations were done in a room saturated with water vapor. The tissues were dried in an evacuated desiccator over $CaCl_2$ for 48 hours and then to constant weight over P_2O_5. We believe that this procedure gives more reliable results than drying in an oven, but as a check on our results, the tissues were kept in an oven at 105°C. after removal from the desiccator until they again reached a constant weight. There was a further loss of weight in the oven of approximately 1 per cent for all the tissues, which may have been due to loss of other substances than water (5). It was found that for comparative purposes the results obtained by the two procedures were the same.

Results. When the animals were allowed to eat *ad libitum* those on the B-free diet consumed much less food over the entire experimental period than the controls on the same ration with yeast added. The males consumed the equivalent of 972 calories and the females 841 on the B-free diet while the intake of the controls was 1587 and 1330 calories, respectively. The experimental males lost 23 per cent of their original body weight and the females 22 per cent although they were at the age when normally there is rapid growth. The control males on the other hand gained 39 percent and the females 32 per cent in body weight.

In the balanced experiments there was a greater loss of weight on the B-free than on the control ration. The males lost 18 per cent of their original body weight and the females 19 per cent on the B-free ration while the control animals

consuming the same amount of food lost 4 per cent and 13 per cent, respectively. Since the caloric intake of the experimental and control groups was the same, it would appear that the absence of the B complex resulted in an impairment of the absorption of the food material. Data on the amount of food absorbed, however, is not available as the feces were not collected for analysis.

The percentage water content of the tissues and of the body obtained by drying in a desiccator is shown in table 1 for both groups of experiments on the two rations. There was no significant difference in the water content of the brain, liver, kidneys and muscle on the B-free and control rations either when food was taken *ad libitum* or when the caloric intake was balanced. The skin and the body of the animals that were permitted to eat *ad libitum* had a higher percentage water content on the B-free than on the control ration with yeast added. When the food intake was equalized, however, there was no significant difference in the

TABLE 1

*Percentage water content of various organs and of the body of albino rats on a vitamin B-free ration and on the same ration with yeast added**

TISSUE	FOOD INTAKE AD LIBITUM				CALORIC INTAKE EQUALIZED			
	Males		Females		Males		Females	
	B-free ration	Same ration plus yeast	B-free ration	Same ration plus yeast	B-free ration	Same ration plus yeast	B-free ration	Same ration plus yeast
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Brain	77.6	77.9	77.5	77.8	77.4	77.5	77.6	77.7
Liver	69.1	69.3	70.6	69.2	67.8	69.0	70.3	70.5
Kidneys	75.9	75.5	76.5	75.9	75.5	75.2	76.4	76.3
Gastrocnemius	74.4	74.6	74.0	74.3	74.1	75.1	74.4	74.5
Blood	78.6	79.8	78.6	79.9	78.5	78.7	78.6	78.3
Skin	62.8	59.0	63.8	55.3	62.7	64.1	63.8	61.7
Body	65.9	60.4	64.7	58.8	66.4	66.5	66.1	65.6

* Each value in the table is an average of 8 experiments.

percentage of water in the entire body on the experimental and control rations. There was a small difference in the water content of the skin. It will be observed, however, upon comparison of the experiments on the ration with yeast added that the percentage of water in the skin was considerably higher when the food intake of the controls was restricted in the balanced experiments than with the larger food intake when the animals were allowed to eat *ad libitum*.

The average water content of the blood of all animals was 78.6 per cent on the B-free and 79.9 per cent on the control ration in the *ad libitum* experiments. The numerical difference, although small, was statistically significant. The percentage was lower on the B-free ration in 14 out of 16 experiments. The reduction in the percentage water in the blood of the experimental animals was obviously due to the smaller food intake for, when the caloric intake was equalized, the blood of the animals on the B-free ration contained 78.6 per cent water and that of the controls 78.5 per cent.

Discussion. The larger percentage of water in the body on the B-free than on the control ration when the animals were allowed to eat *ad libitum*, must be attributed to the small food intake and not to the absence of the B complex. This becomes obvious upon comparison of these experiments with those in which the caloric intake of the experimental and control animals was equalized. In the latter experiments the percentage of body water was the same on the two rations although the control ration contained a liberal amount of the B complex.

In grinding the body all the skin except the small portion that had been removed for drying was included. The values for the percentage water content of the body in table 1 are consequently affected by the amount of water in the skin which was different on the two rations. It was shown, however, by calculations of the water content of the body exclusive of the skin, that the differences in the percentage water of the body on the two rations with *ad libitum* feeding can not be explained by differences that obtained in the water content of the skin. When allowance was made for the total amount of water in the skin on the basis of the ratio of the weight of the skin to body weight, there remained a large difference in the percentage water of the body on the two rations which was statistically significant. The ratio of the weight of the skin to body weight was determined by weighing the entire skin and body of other rats kept under the same conditions as those of the present experiments. Nor could the difference have been due to changes in the water content of the muscle or other organs. As shown in table 1, the percentage of water in the muscle and other organs was the same in the experimental and control animals. It is suggested that the difference in the water content of the body on the two rations may have been related to a diminution in the fat in the body. There were no analyses made of the fat content of the body but it was apparent upon gross examination of the animals allowed to eat *ad libitum* that there was less fat in the body on the B-free than on the control ration. The experimental animals had lost an appreciable amount of weight and were emaciated. The percentage of water in the skin has been shown to vary inversely with the fat content (6), and it is not unreasonable to suppose that there probably exists a similar relationship between the percentage of fat and of water in the body.

The larger percentage of water in the skin on the B-free than on the control ration with *ad libitum* feeding must be attributed, as in the case of the body, to the small food intake resulting from a deficiency of the vitamin B complex and not directly to the lack of the complex itself. The higher water content was probably related to a decrease in the fat content. In another series of similar experiments on 8 female rats which are not included in table 1, the percentage water in the skin on the B-free ration was 61.5 while that of the controls was 52.7; the fat content was 7.4 and 22.4 per cent, respectively.

It is of interest to note that the sex difference in the water content of the skin which has been reported in previous studies (6) did not obtain on the B-free ration but was observed when the animals were fed the same ration with yeast added. These results would suggest that the different percentage water content of the skin of the two sexes may be due to differences in the metabolic processes in the

tissue and that these processes do not function normally in the absence of the B complex.

From their observations on the effects of induced thiamin deficiency in human subjects, Williams and his associates (3) were led to question whether the lack of thiamin is responsible for edema and the other classic features of beri-beri. The present experiments would suggest that the edema encountered in beri-beri may not be the direct effect of a deficiency of any of the fractions of the vitamin B complex.

CONCLUSIONS

The water content of the body of the albino rat was not directly affected by deprivation of the vitamin B complex.

The percentage of water in the body of animals fed a B-free ration was higher than that in the controls on the same ration to which yeast was added. This, however, was not due to the lack of the B complex but to the reduction in food intake resulting from the vitamin deficiency. When the food intake was equalized the water content of the body on the two rations was the same.

The water content of the skin like that of the body was greatly increased as a result of the diminution in food intake consequent to B complex deficiency. Conversely, the percentage of water in the blood was lowered, although only to a slight extent, by the reduction in food intake.

There were no significant differences in the amount of water in the brain, liver, kidneys and gastrocnemius muscle on the B-free and control rations.

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HYPOGLYCEMIC EFFECTS OF GROWTH HORMONE IN FASTING HYPOPHYSECTOMIZED RATS[1]

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Received for publication December 7, 1943

Attempts are being made in this institute to investigate the rôle played by anteropituitary hormones in the mechanism regulating carbohydrate metabolism. Recently experiments were reported on the action of purified growth hormone practically free of other "contaminating" hormones, on pancreatic insulin content in normal rats (1), on muscle glycogen in normal and hypophysectomized rats (2), on urinary sugar excretion in partially depancreatized rats (3), and on the respiratory quotient in normal and hypophysectomized rats (4). The present communication deals with the effects of purified growth hormone on blood sugar in fasting hypophysectomized rats.

METHODS. Immature female rats were hypophysectomized at 26 to 28 days of age. After a postoperative period of approximately two weeks, the animals were fasted for 17 to 18 hours, and then one half of the rats were injected with growth hormone. Simultaneously with each experimental animal, a rat of the group which served as control was injected with saline. Within 1 to 3 hours almost all the rats injected with growth hormone exhibited convulsions. Immediately upon manifestation of such symptoms, each animal was anesthetized (sodium amytal), and a blood sample was taken from the vena cava for sugar analysis. Simultaneously with each experimental animal, a control rat was anesthetized and its blood obtained in the same way. The animals were then sacrificed and the region of the sella turcica examined carefully in order to check completeness of the hypophysectomy. Blood sugar was determined according to a modification of Benedict's method (2).

The growth hormone preparations were cysteine-treated globulin fractions, all except one further purified by fractionation by pH variation (5). Their growth hormone potencies varied from approximately 25 to 200 $[GU]^{10}_{HR}$*. No gonadotropic or adrenocorticotropic and only a trace of thyrotropic hormones were detectable in these preparations when they were injected into hypophysectomized rats at a total dose of 5 mgm. (4 day test). Such preparations have always been found free of lactogenic hormone, when injected into immature pigeons at a total dose of 20 mgm. (4 day test, systemic injections).

RESULTS. Table 1 summarizes the results of 4 experiments using a total of 86 rats. The average blood sugar levels of hypophysectomized controls (when fasted for approximately 20 hrs.) were found between 40 and 47 mgm. per cent.

[1] Aided by grants from the Board of Research of the University of California, the National Research Council Committee on Research in Endocrinology and the Rockefeller Foundation, New York City.

* $[GU]^{10}_{HR}$ = growth hormone units per milligram, 10 day test in hypophysectomized rats (6).

No hypoglycemic symptoms such as torpor or convulsions were manifest in these controls, except in the case of a very few individuals which had unusually low blood sugar values due to the fasting alone. When a single dose of growth hormone was administered to such hypophysectomized rats after a fasting period of about 18 hours, the majority if not all of these animals exhibited within 1 to 3 hours typical hypoglycemic symptoms such as convulsions or torpor. Their average blood sugar values were considerably lower than those of the controls (by about 40–60 per cent). The difference was highly significant. The correlation between blood sugar levels and incidence of hypoglycemic symptoms was very good; only when the blood sugar dropped below about 30 mgm. per cent were convulsions exhibited.

TABLE 1

Exacerbation of the hypoglycemia in fasting hypophysectomized rats by the administration of growth hormone

DOSE GROWTH HORMONE	NUMBER OF RATS	INCIDENCE OF HYPOGLYCEMIC SYMPTOMS		BLOOD SUGAR		
		Convulsions	Torpor	Mean value	Decrease and standard error	Decrease as % of control value
GU^{10}_{HR} *		*per cent*	*per cent*	*mgm. per cent*	*mgm. per cent*	
900	11	100	0	21	26 ±2.3	55
0	11	0	9	47		
900	11	91	9	16	24 ±6.5	60
0	10	0	0	40		
260	14	71	0	28	18 ±5.6	39
0	8	0	13	46		
80	10	70	30	26	17 ±5.8	40
0	11	27	9	43		

* GU^{10}_{HR} = growth hormone units, 10 day test in hypophysectomized rats.

Further evidence for the convulsions being a consequence of the low blood sugar level was obtained in the following way: A group of hypophysectomized rats was fasted and injected with growth hormone as described above. As soon as the animals showed convulsions, 1 cc. of a 20 per cent glucose solution was injected, and it was found that the symptoms disappeared immediately.

Since quantities as high as 4 mgm. of growth hormone were injected in the single dose employed, it was considered important to exclude the possibility that the observed hypoglycemia was a consequence of a non-specific "protein reaction." For this reason, 5 mgm. of commercial casein (the portion soluble at approximately pH 8), were injected under conditions as described above to each of a group of 16 rats. When compared with their untreated controls, these animals had practically identical blood sugar concentrations, and they did not show any different symptoms.

The only anteropituitary hormone found to "contaminate" the growth hor-

mone preparations here employed was thyrotropic hormone. Although the quantity of thyrotropic hormone present in these preparations was less than one per cent by weight, it was considered important to investigate whether thyrotropic hormone or thyroxin could exert a blood sugar-lowering effect under these circumstances. For this purpose, thyroxin was injected instead of growth hormone at a dose level of 10 micrograms to 8 hypophysectomized rats under conditions otherwise identical with those described above. In contradistinction to the effects obtained with growth hormone, convulsions were not observed within 3 hours after the injection of thyroxin, and no significant difference in blood sugar values was found between experimental and control rats. It is obvious, therefore, that the depression of blood sugar cannot be attributed to the insignificant contamination of the growth hormone preparations with thyrotropic hormone.

Discussion. The experimental results reported above demonstrate that growth hormone causes a further depression of the blood sugar in fasting hypophysectomized rats. It has already been observed that crude anteropituitary preparations may cause a lowering of the blood sugar level in animals with an intact pancreas (7, 8, 9), and this action was tentatively attributed to a stimulation of the islet tissue, causing an increased insulin secretion (7, 10, 11). The experiments reported here suggest that the growth hormone present in these crude pituitary extracts may have been one of the factors (if not the only factor) responsible for the reduction of the blood sugar concentration. The concept is in agreement with the researches of Mirsky (12) and of Gaebler (13) and their coworkers, all of which emphasized the possible mediation of insulin in the action of the growth hormone in provoking protein anabolism.

SUMMARY

Purified growth hormone was injected into hypophysectomized rats which had been fasted for approximately 18 hours. Within 1 to 3 hours, typical hypoglycemic symptoms were observed, and the blood sugar level of these animals was invariably depressed further than that of untreated controls fasted for the same period.

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EFFECTS OF ORAL ADMINISTRATION OF THIOURACIL ON THE METABOLISM OF ISOLATED TISSUES FROM NORMAL AND HYPERTHYROID RATS

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Received for publication November 6, 1943

Reports have appeared in recent months to the effect that thiourea and some of its derivatives produce in some animals hyperplasia of the thyroid and hypothyroidism (1–3). Astwood (4) has studied a great number of compounds of the thiourea class and has found thiouracil (2-thio-6-oxypyrimidine) to be the most active member. Clinical experience has shown thiouracil to ameliorate the increased basal metabolism and other manifestations of thyrotoxicosis (5–7).

The experiments to be reported were designed to test the action of thiouracil on organs from normal animals, as well as from those in which a state similar to clinical hyperthyroidism was induced by administration of thyrotropic hormone.

1. *Animals and dosage.* Two main series of experiments were carried out.

Series A. White mature Wistar rats, weighing 250 to 300 grams, on a stock diet of Purina dog chow, were divided into four groups. *Group 1* served as control. *Group 2* received a solution of 0.5 per cent of thiouracil[2] in tap water in the form of its sodium salt as its sole supply of liquid. *Group 3* was injected subcutaneously twice daily with 0.1 cc. of a preparation of thyrotropic hormone.[3] *Group 4* received both thiouracil and thyrotropic hormone.

At intervals of 2 to 3 days, animals from each group were killed and the respiration of their organs was measured as described below.

Series B.[4] Immature white rats, weighing about 35 grams, were divided into groups 1 to 4 equivalent to those of series A, with the only difference that the concentration of thiouracil in the drinking water was 0.25 per cent. The basal metabolism of these rats was followed for a period of 22 days (8). At the end of this time, four animals of each group were killed and the respiration of their organs was studied.

2. *Determination of tissue respiration.* The animals were killed by a blow on the head and the thyroid, adrenals, liver and diaphragm were excised. The liver was sliced with a razor in the usual way. Thin strips of diaphragm muscle, near its abdominal attachment, were used without further slicing. The whole thyroid and both adrenal glands were freed completely from surrounding tissue and cut into thin slices by means of small sharp scissors.

[1] Fellow of the Commonwealth Fund.

[2] The thiouracil used in these experiments was furnished by the Lederle Laboratories, Inc., Pearl River, N. Y.

[3] Thyrotropic hormone (Antuitrin-T) was furnished through the courtesy of Parke, Davis and Co., Detroit, Mich.; 1 cc. of this preparation contains approximately 50 Junkmann-Schöller units.

[4] This series is the same as series B of Bissell and Williams (8).

The rate of oxygen uptake of these tissue preparations was measured in Warburg respirometers with conventional flasks (volume approximately 12 cc.) in 3.0 cc. of the "phosphate medium", the composition of which is shown in table 1.[5] This medium has been compared with Ringer's solution and other media, and found to yield consistent, reproducible and stable rates of oxygen uptake. The respiration of rat liver slices in this medium often remains constant for as long as 4 hours. In the center cup of each flask was placed 0.3 cc. of 20 per cent KOH. The manometers were shaken at a rate of 100 to 120 oscillations per minute at 38°, flushed with oxygen for 10 minutes, and readings were taken at intervals of 10 minutes for 2 hours. At the end of this period, the tissue slices were removed from the flasks, rinsed in distilled water, dried at 105° for 24 hours, and weighed.

The amount of thyroid tissue which could be obtained from one rat, especially when dealing with normal animals, was too small to permit accurate measurement of its oxygen uptake under the above conditions. To overcome this

TABLE 1

Composition of phosphate medium used in the measurement of oxygen consumption of rat tissues

SALT	CONCENTRATION OF STOCK SOLUTION	AMOUNT REQUIRED FOR 100 CC.	FINAL CONCENTRATION
	mM. per liter	*cc.*	*mM. per liter*
NaCl	154	80.9	124.6
KCl	154	2.6	4.0
$CaCl_2$	104	1.0	1.0
$MgCl_2$	104	0.5	0.5
Phosphate buffer, pH 7.4	67	15.0	10.0
Glucose			(0.2%)

difficulty, small vessels of approximately 5 cc. capacity[6] were used, with 1.0 cc. phosphate medium in the main flask and 0.1 cc. of 20 per cent KOH in the center cup. Since these vessels were not equipped with an outlet at the sidearm, air instead of oxygen was used as the gas phase. When the oxygen uptake was increased (due to thiouracil or thyrotropic hormone), ordinary Warburg vessels and oxygen were used for the measurement of thyroid respiration in series A. The results obtained with one technique are comparable to those obtained with the other. The respiration of thyroid tissue is the same in air as in oxygen under our conditions, and variations in the rate of shaking from 70 to 140 oscillations per minute have no effect on the rate of oxygen uptake of thyroid slices as measured by either procedure.

All results are expressed in the usual way as $-QO_2$ = cmm. O_2 consumed per hour per mgm. dry weight of tissue.

[5] The composition of this medium was suggested to one of us (B. J. J.) by Dr. A. B. Hastings, Dept. of Biological Chemistry, Harvard Medical School.

[6] We wish to thank Dr. B. S. Gould of the Mass. Institute of Technology for the loan of these vessels.

EXPERIMENTAL. 1. *Series A.* a. *Thyroid.* The changes in QO_2 of the thyroid glands of rats which were given thyrotropic hormone, thiouracil or both are shown in figure 1A. Thyrotropic hormone exerted a stimulating action upon thyroid respiration which became noticeable a few days after beginning its use. The effect of thyrotropic hormone was not studied beyond the 20th day; it has been our experience that after about 3 weeks of hormonal treatment there is a reversion to normal, both in the basal metabolic rate of rats (8) and in the QO_2 of their tissues. This is possibly due to an antihormone effect.

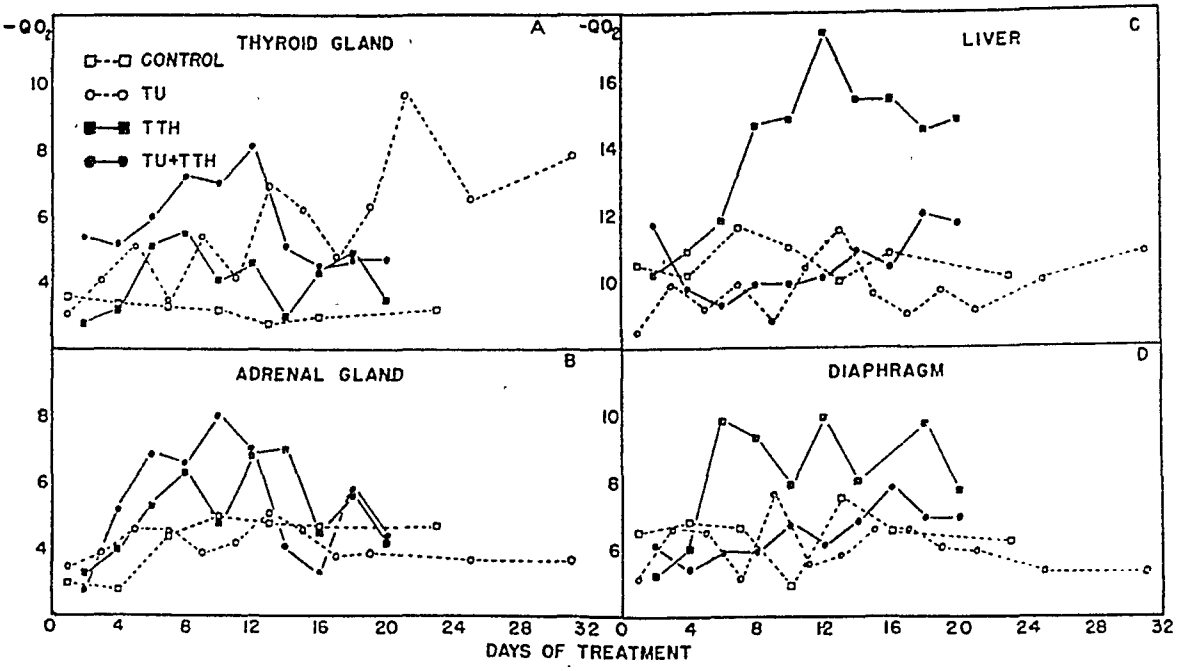

Fig. 1, A. Effect of administration of thiouracil, thyrotropic hormone or both on the oxygen consumption of rat thyroid glands.

Fig. 1, B. Effect of administration of thiouracil, thyrotropic hormone or both on the oxygen consumption of rat adrenal glands.

Fig. 1, C. Effect of administration of thiouracil, thyrotropic hormone or both on the oxygen consumption of rat livers.

Fig. 1, D. Effect of administration of thiouracil, thyrotropic hormone or both on the oxygen consumption of rat diaphragm muscles.

Thiouracil administration, during the first 10 to 12 days, increased the QO_2 of rat thyroids to the same extent (about 50 per cent) as did thyrotropic hormone. After this time, stimulation became much more marked and reached levels of more than 3 times the normal after about 3 weeks of continuous administration of the drug.

When both thiouracil and thyrotropic hormone were given to rats, the QO_2 of their thyroid glands during the first 2 weeks of administration was higher than in those which received either drug alone. The stimulation made its appearance as soon as treatment was instituted. This stands in contrast to the effects of either thiouracil or thyrotropic hormone, which caused an increase in thyroid respiration only after an initial lag period of 2 to 4 days. After about

12 days the stimulation became less marked, and during the third week the values obtained were approximately those found when the hormone alone was administered.

A rather close parallelism was found to exist between the QO_2 and weight changes of the thyroids. The weight, as well as the respiration per unit of weight, was increased by either drug to about the same extent during the first $1\frac{1}{2}$ to 2 weeks, more so when both drugs were administered. The rapid increase in weight of the thyroids of rats given thiouracil seemed to precede that in QO_2 by a few days.

b. *Adrenals.* Figure 1B shows the effect of thiouracil and thyrotropic hormone, singly or in combination, on the oxygen consumption of rat adrenal tissue. Thiouracil alone, during the whole period of observation, did not affect the

TABLE 2

Effect of oral administration of thiouracil, subcutaneous injection of thyrotropic hormone, or both on the oxygen uptake of rat organs (series B)

Results are expressed in terms of $-QO_2$ (cmm. O_2 consumed per hour per mgm. dry weight) and represent the averages of 4 determinations. Figures in parentheses indicate the per cent increase above normal.

TREATMENT	LENGTH OF TREATMENT	THYROID	ADRENAL	LIVER	MUSCLE
	days				
Control		2.2 ± 0.4	2.4 ± 0.1	10.8 ± 0.9	7.0 ± 0.8
Thiouracil	26–27	7.9 ± 0.5 (259)	2.5 ± 0.3 (4)	9.4 ± 0.2 (−13)	6.8 ± 0.8 (−3)
Thyrotropic hormone	22–24	5.0 ± 0.8 (127)	2.9 ± 0.4 (21)	14.1 ± 1.2 (31)	10.2 ± 1.3 (46)
Thyrotropic hormone and thiouracil	24–25	8.8 ± 1.4 (300)	5.0 ± 1.5 (117)	9.8 ± 0.6 (−9)	6.1 ± 1.1 (−13)

respiration of this organ to any extent outside the limits of experimental error. Thyrotropic hormone, with or without simultaneous administration of thiouracil, caused a small transient rise in the oxygen consumption during the second week of the experiment.

c. *Liver and diaphragm muscle.* Figure 1C and D indicate the effects of thyrotropic hormone and thiouracil on the QO_2 of liver and muscle, respectively. The respiration of both organs was markedly stimulated by thyrotropic hormone. The simultaneous administration of thiouracil reduced their respiration to normal or slightly subnormal levels. Thiouracil alone had little or no effect on the oxygen consumption of liver or muscle at any time during the four weeks of the experiment.

2. *Series B.* In table 2 are recorded the average QO_2 and its mean deviation of the thyroid, adrenals, liver and diaphragm muscle of rats which had been treated for 22 to 27 days with thiouracil, thyrotropic hormone, or both, together with controls. Four rats were used from each group. The results of this series are in accord with those obtained in the previous one. The respiration of the

thyroid was stimulated by either drug, and a combination of both had a more powerful action than either one alone. Neither drug had much of an effect on the respiration of the adrenal glands; administration of both caused a somewhat greater increase than was found in series A. Finally, liver and diaphragm respiration was increased by thyrotropic hormone, while thiouracil, in the absence or presence of administered hormone, did not influence their respiration to an extent beyond the limit of accuracy of the measurements.

Discussion. The studies of Richter and Clisby (1), Mackenzie and Mackenzie (2), and of Astwood *et al.* (3, 4) have demonstrated that thyroid hyperplasia can be induced by thiourea and some of its derivatives. This effect can be counteracted by feeding thyroxine or thyroid powder, or by hypophysectomy, but not by iodine, or by diiodotyrosine except in very large doses. Compounds of the thiourea series are also effective in lowering the basal metabolism in animals and in patients suffering from hyperthyroidism according to Astwood *et al.* (3, 5) and Williams and Bissell (6–8).

On the basis of these histological and metabolic findings, Astwood *et al.* (3) have advanced the hypothesis that the mechanism of action of thiourea derivatives is to inhibit the formation of thyroid hormone. An exhaustion of the stores of thyroid hormone results in a fall in the basal metabolic rate. Presumably an increased amount of thyrotropic hormone is produced which leads to hyperplasia of the thyroid gland. The increased production of thyroid hormone in hyperthyroidism might be inhibited in a similar way by thiouracil.

The results presented in this study seem to support the above hypothesis for the action of thiouracil both in normal animals and in those in which hyperthyroidism was induced by the administration of thyrotropic hormone. There is a marked contrast in the reaction to this drug of the thyroid gland on one hand, and of liver and muscle on the other. The last two tissues can be regarded as "metabolic end-organs," their rate of metabolism being, in part at least, governed by the rate of production of thyroid hormone.

Thyrotropic hormone alone caused an increase in the rate of oxygen consumption of all organs studied; this effect is well-known and requires no further comment. The hyperplasia of the thyroid gland under the influence of thiouracil was reflected not only by its increase in dry weight, but also by its raised metabolism per unit weight. For the first 1½ to 2 weeks, the increase in both was about 50 to 100 per cent, and it may be assumed that small amounts of thyroid hormone were either still being produced, or that remaining reserves were being mobilized. After this period hyperplasia became more marked and was followed within a few days by a sharp increase in the metabolism as reflected by the consumption of oxygen.

A combination of both drugs might be expected to produce a potentiation of the effects of either drug alone, since the presumably increased production of thyrotropic hormone, secondary to the administration of thiouracil, is here supplemented by an injection of this hormone. This is confirmed by our experimental results. On the same basis, the absence of a lag period in the stimulation of thyroid respiration when both drugs were given is presumably due to the

obviation of the slow "gearing up" of the rate of thyrotropic hormone production in the pituitary when thiouracil alone is administered.

The respiration of liver and muscle, on the other hand, was little influenced by thiouracil administration. Moreover, their metabolism, when increased by injection of thyrotropic hormone, was reduced to normal values when thiouracil was given simultaneously. Since these tissues can be considered to be representative of those contributing to the bulk of oxygen uptake of an intact animal, the above results may be compared with similar ones on normal and hyperthyroid animals (3, 8) and patients (5–7).

The results obtained with the adrenal glands are more difficult to explain. The effects seemed to be intermediate between those on the thyroid, and on liver and muscle. Thiouracil or thyrotropic hormone alone had very little effect on adrenal respiration, beyond a temporary rise with the latter drug, while a combination of both produced a small increase in mature, and a more marked one in immature, animals. It may be thought that thiouracil produces an increased production not only of thyrotropic but also of other pituitary hormones, including the adrenotropic. However, if this were so, thiouracil alone should be expected to increase the respiration of adrenal glands too.

SUMMARY

Rats were given thiouracil in the drinking water, thyrotropic hormone by subcutaneous injection, or both. At frequent intervals the QO_2 of thyroid, adrenals, liver and diaphragm muscle obtained from these groups was measured manometrically and compared with those of untreated animals.

The respiration of the thyroid gland is increased by both drugs; the effect of their combination is greater than that of either alone. This increase is much more than can be accounted for by the concomitant hyperplasia of the gland.

The adrenal glands show little or no change in their respiration under the influence of either thiouracil or thyrotropic hormone alone, while their combination produces an increase in young but not in mature animals. Liver and muscle show an increased metabolism when thyrotropic hormone is administered; this effect is abolished by simultaneous treatment with thiouracil. Thiouracil alone does not influence the metabolism of these organs appreciably.

The results are discussed in the light of the hypothesis of a compensatory hypertrophy of the thyroid gland due to the inhibition by thiouracil of the formation of thyroid hormone.

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SURVIVAL OF REFLEX CONTRACTION AND INHIBITION DURING CORD ASPHYXIATION

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Received for publication November 29, 1943

In previous papers (10, 9) the course of recovery of reflex activity after asphyxiation of the spinal cord for periods ranging from 25 to 65 minutes has been studied. It was found that periods of asphyxia which prevent recovery of the flexion reflex may permit the return of tendon reflexes, and that the tendon reflexes can also return when asphyxiation has completely destroyed reflex inhibition. From these experiments the conclusion was drawn that tendon reflexes are more resistant to asphyxia than the flexion reflex and reflex inhibition.

It seemed of interest to compare the above results, which were based on the ability of reflexes to recover from asphyxiation, with the resistance of the same reflexes to a developing asphyxia of the spinal cord. In the present investigation the survival times of the kneejerk, the flexion reflex and of the inhibition of the kneejerk during asphyxiation of the cord were compared.

METHODS. The spinal cord in cats was asphyxiated by forcing Ringer solution into the isolated caudal part of the dural cavity under a pressure higher than the blood pressure (24–26 cm. of mercury). On the day preceding the experiment the dura was ligated at Th 10–12, severing the spinal cord at that level. The experiment itself was performed in a room kept at 37–38° and the Ringer solution thus entered the dural cavity at body temperature. Light nembutal narcosis was used.

In one series of experiments the survival times of the kneejerk and of the flexion reflex were compared. The femur of one hind leg was fixed on a board with screws. The kneejerk was elicited by tapping the quadriceps tendon with an electromagnetic hammer resembling the apparatus described by Johnson (3). The movements of the shank were recorded on a smoked drum. The shank of the other hind leg was fixed with screws and the tendon of the m. tibialis anterior was connected with a lever, writing on the same drum. The flexion reflex was elicited by short faradic stimulations of the homolateral n. peroneus superficialis. The stimuli for kneejerk and flexion reflex were given alternately by means of a set of mechanically driven contacts at intervals of 2.5 to 3 seconds.

In another series of experiments the survival times of the kneejerk and of reflex inhibition were compared. In addition to the ligation of the dura on the day preceding the experiment, the peroneal and tibial nerves were severed in the popliteal cavity, the nerve branches for the hamstring muscles were cut and the m. biceps femoris was transected. These operations were carried out with aseptic precautions. The next day the animal was prepared for recording of the kneejerk as described above. This reflex was elicited at intervals of 2.5 to

3 seconds and alternate kneejerks were inhibited by short faradizations of the peroneal or of the tibial nerve which were timed to precede and accompany the kneejerk stimuli.

The effect of cord asphyxiation on kneejerk and flexion reflex. Figure 1A is a typical curve showing the result of cord asphyxiation on kneejerk and flexion reflex. The application of pressure to the dural cavity itself has no obvious effect, but after 10 to 15 seconds contractions of the quadriceps, as well as those of the m. tibialis, increase markedly. After reaching a maximum the reflex contractions decline and soon cannot be elicited any more. The increase of the reflex response during the development of asphyxia varies, usually it is marked as shown in figure 1A, but in some experiments it is hardly present.

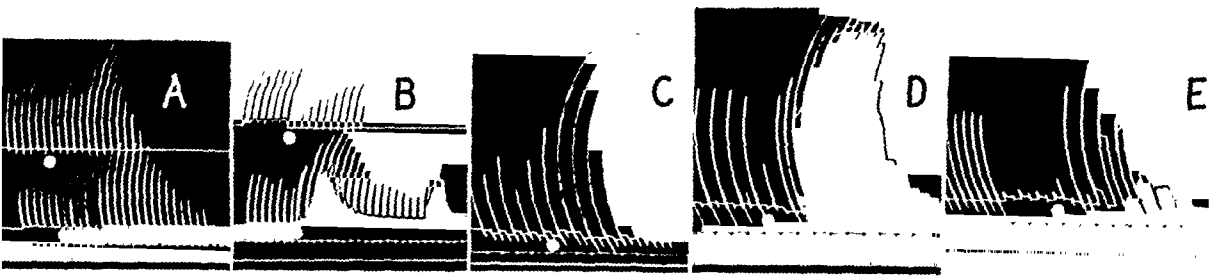

Fig. 1 A and B. Kneejerk (upper line) and flexion reflex (second line) during cord asphyxiation which was started at the white spot. The third line shows the short faradic stimulations causing the flexion reflex. The lowest line is a 1 sec. time signal. The figures A and B are of different animals.

Fig. 1 C shows the influence of cord asphyxiation, beginning at the white spot, on the inhibition of the kneejerk (upper line). The second line shows the faradic stimuli which inhibit alternate kneejerks. The lowest line is a 1 sec. time signal. The coil distance of the inhibitory stimulus was 10 cm., whereas a stimulus at a coil distance of 14 cm. caused just complete inhibition.

Fig. 1 D and E. The meaning of the lines is the same as in figure 1 C. The inhibitory stimulus in D caused just complete inhibition (coil distance 10.5 cm.). In E the inhibitory stimulus was increased considerably (coil distance 5 cm.).

Not always, however, are the changes of the reflex activity so simple during the development of asphyxia. In a few experiments the m. tibialis anterior contracted when pressure was applied to the dural cavity and the next few kneejerks were greatly reduced (fig. 1B). This probably results from the sudden extension of the dura and of the joints and ligaments of the lower part of the spinal column which, acting as a strong stimulus, causes reflexly a contraction of the anterior tibial muscle and an inhibition of the kneejerk. The inhibition of the kneejerk occurred often without an accompanying contraction of the tibialis muscle.

Usually the lever returned to the base line after each contraction of quadriceps or tibialis muscle during the entire period of survival (fig. 1A). Sometimes, with the increase of the reflex response, a tonic contraction of the muscle developed upon which the reflex contractions were superimposed. This is shown for the m. tibialis anterior in figure 1B, for the quadriceps in figure 1D.

In a few experiments a contraction of a tonic character developed in the m.

tibialis either during the final reflex contractions (fig. 1B) or 5 to 10 seconds after the last reflex contraction was observed. This contraction was of only a few seconds' duration. When immediately after it had disappeared the pressure in the dural cavity was released, the renewed oxygenation of the cord sometimes caused a similar contraction of the tibialis muscle.

Finally, during the period of increased reflex response in the beginning of asphyxiation, the kneejerk stimulus sometimes caused crossed flexion. This can be seen in figure 1B as small tibialis anterior contractions between the larger tops caused by the faradization of the n. peroneus superficialis.

The above phenomena can occur alone or in combination. They may be partly due to the experimental circumstances, but are probably to a great extent individual reaction types of the animal.

It was found that when a rest period of 10 to 15 minutes was allowed after each asphyxiation and if the pressure application was not prolonged beyond the abolition of reflex activity, asphyxiation could in general be repeated without

TABLE 1

Survival periods of kneejerk and flexion reflex (in sec.) during a series of cord asphyxiations with 15 minutes intervals

	NUMBER OF ASPHYXIATION						
	I	II	III	IV	V	VI	VII
Survival period of kneejerk	46	34	35	41	42	36	39
Survival period of flexion reflex	59	54	51	56	54	59	57

much change in the survival time of kneejerk and flexion reflex. This is demonstrated in table 1. In some experiments, however, the survival time had a tendency to decrease in the course of such an experiment. The survival times of kneejerk and flexion reflex have been determined 48 times in 9 animals. With five exceptions, the survival time of the flexion reflex has been longer than that of the kneejerk. The survival time of the kneejerk varied between 15 and 80 seconds, and that of the flexion reflex between 22 and 61 seconds. The average survival period of the kneejerk was 31 $\pm$ 0.9 seconds, and the average for the flexion reflex was 41 $\pm$ 0.8 seconds. The survival time of the flexion reflex thus seems to be significantly longer than that of the kneejerk.

The effect of cord asphyxiation on reflex inhibition. Figure 1C shows the effect of cord asphyxiation on reflex inhibition with a stimulus stronger than necessary for complete inhibition of the kneejerk. As can be seen, the inhibition of the alternate kneejerks continues undisturbed during asphyxiation as long as the kneejerk can be elicited, and the survival time of reflex inhibition thus must be at least as long as that of the kneejerk. If the inhibitory stimulus is made only just strong enough to cause complete inhibition, there is often an escape of the inhibited kneejerk during the period of increased response in the beginning of cord asphyxiation; when the reflex response decreases, inhibition becomes again complete.

In one animal it has been possible to examine the effect of the inhibitory stimulus on the tonic contraction of the quadriceps which is sometimes observed during the increased reflex response in the beginning of cord asphyxiation. In the curve of figure 1D the inhibiting stimulus was just sufficient to cause complete inhibition of the kneejerk. After the application of pressure to the cord the first uninhibited kneejerk is small and is thus probably inhibited by the stimulus of the pressure. Then the inhibitory stimulus becomes insufficient and each of the subsequent kneejerk stimuli causes a response. Soon the tonic contraction builds up and is hardly influenced by the inhibitory stimuli. When the inhibitory stimulus was increased in the same animal, quite a different curve was obtained. In the curve of figure 1E, the inhibiting stimulus was so strong that its effect was still sufficient, after 2.5 to 3 seconds, to prevent the reflex response of the "uninhibited" kneejerk. However, when the cord was put under pressure, the uninhibited kneejerks escaped and became quite large. The tonic rests of these reflex responses are inhibited by each of the following inhibitory stimuli. A tonic contraction also developed in the quadriceps when the cord in this animal was asphyxiated without any kneejerk stimulus being given. This tonic contraction could be inhibited by strong faradic stimulation of the n. tibialis. The fact that the tonic contraction during the period of increased reflex response can be inhibited is indicative of its reflex nature.

Later effects of cord asphyxiation. It has been found frequently that 5 to 10 minutes after the onset of cord asphyxiation a contraction of a tonic character develops in the quadriceps and gastrocnemius-soleus muscles. This contraction usually reaches a maximum about 15 minutes after the beginning of asphyxiation, and disappears after 20 to 25 minutes.

In 10 experiments the action potentials accompanying this contraction were recorded, using a Matthews oscillograph and silver wires placed in the quadriceps and triceps muscles. Before asphyxiation, some electrical activity was usually present which could be increased by stretching the muscle. In the period between 1 and 3–5 minutes after the beginning of asphyxiation, no potentials could be led off. After this period spikes appeared, in most cases first in the triceps group but later also in the quadriceps muscle. They grew in size and frequency, reaching a maximum 15 to 20 minutes after the beginning of asphyxia, and disappeared after 25 to 30 minutes. Attempts to influence these action potentials by stretching the muscle generally failed.

Discussion. An increased reflex response of the kneejerk as well as of the flexion reflex has been described by several authors (6, 4, 2, 12, 1, 8) during the initial stages of asphyxia or anoxia of the spinal cord produced in various ways. Porter (7) found no decrease of the threshold of the flexion reflex under these circumstances but in a later paper with Blair and Bohmfalk (8) using the small tenuissimus muscle, he could show in the beginning of the cord asphyxiation a spread of activity to motor units which previously were not active. In most of the present experiments the increased reflex response has been observed. Sometimes, however, the inhibition of the kneejerk caused by the application of pressure to the dural cavity interferes with the increased reflex response to such

an extent that the resulting reflex contractions remain smaller during the development of asphyxia than before.

When the survival period during the development of cord asphyxiation is taken as the criterion, the kneejerk is less resistant than either the flexion reflex or reflex inhibition. However, when the recovery of these reflexes from relatively long periods of asphyxiation is considered, the kneejerk is the more resistant (10, 9). This may indicate that the mechanism involved in the suppression of reflex activity during the development of cord asphyxiation differs from that determining the destruction of reflex activity by long periods of asphyxiation. Evidence has been presented (11) that the determining factor for the recovery from asphyxiation lies in the ability of the perikarya not only to recover their function, but to remain alive. It is likely that the failure of kneejerk and flexion reflex during the development of cord asphyxiation is due to a less severe change, probably at the synapse.

The nature of the sustained contraction observed between 5 and 25 to 30 minutes after the beginning of cord asphyxiation is not clear. Also, the place where the impulses causing this contraction originate is uncertain. They may not even be of central origin, but may be generated in the ventral roots, since asphyxiation has been shown to produce spontaneous discharges in mammalian nerve (5).

SUMMARY

The periods of survival of the flexion reflex, the kneejerk and the inhibition of that reflex, have been determined during asphyxiation of the spinal cord. The flexion reflex survived cord asphyxiation for a longer period than the kneejerk. Usually an increased reflex response was observed in the beginning of asphyxiation. Inhibition of the kneejerk has been demonstrated as long as that reflex could be elicited.

In many experiments, three to five minutes after the start of cord asphyxiation, a contraction of a tonic character developed in the quadriceps and gastrocnemius-soleus muscles. After reaching a maximum, this contraction disappeared 20 to 25 minutes after the beginning of cord asphyxiation.

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THE EFFECT OF HUMAN PLASMA ON THE VENOPRESSOR MECHANISM[1]

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Received for publication October 5, 1943

The effect of plasma on the venopressor mechanism was first brought to our attention by the observation of an intramuscular pressure reading over 150 mm. water and a venous pressure of 31 cm. water, following the administration of 3 units (750 cc.) of human plasma, in the treatment of severe postoperative depression.

Increments of pressure in the biceps brachii muscle, over 90 mm. water and up to 116 mm. water are uncommon (1).

METHODS. Eight patients were studied for the effect of human plasma on the venopressor mechanism. Three were normal individuals to whom plasma was administered for reasons other than the treatment of shock. Two patients were in a semi-comatose condition following head injuries, who did not have evidence of peripheral circulatory failure. Three patients had undergone the following surgical procedures respectively: 1, thoracoplasty; 2, exploration of the bladder following traumatic rupture of the prostatic urethra; 3, exploration of the abdomen for an abscess of the liver.

In all instances but one, 3 units of plasma (750 cc.) were administered. In the one, only a single unit (250 cc.) was given. This patient served as a control for two factors. These were *a*, the effect of the administration of a single unit of plasma over a period of 90 minutes, and *b*, the effect of repeated injections of small amounts of saline on the readings of intramuscular pressure, with the needle in situ in the muscle for 90 minutes. We previously reported that the procedure was accurate within 10 mm. water for long periods of time (1). The plasma was administered by gravity flow, without the use of pressure to speed the rate of infusion. The plasma bottle was approximately 4 feet above the vein.

Intramuscular pressure and venous pressure were measured by the gravity flow method (2). The venous pressure was measured in the antecubital vein and the intramuscular pressure in the homo-lateral biceps brachii muscle. The limitations of the method, and the errors in consecutive readings for intramuscular pressure (10 mm. water) and for venous pressure (1 cm. water) have been discussed elsewhere (1, 2, 3, 4).

Observations of intramuscular pressure were made at 5 minute intervals. The instrument used in this study was constructed of materials at hand. Spinal manometers with a 1 mm. bore mounted on a vertical base-board were used as

[1] We are indebted to our former Commanding Officer, Captain (now Rear Admiral) J. J. A. McMullin (MC), USN, whose suggestions and co-operation made this study possible. Lt. M. T. Friedell (MC), USNR, gave valuable assistance in arranging and editing.

manometers. This instrument had certain characteristics different from our original instrument (2) which require further description.

The manometer was first filled to the 200 mm. level. A turn of the stopcock permitted the saline to flow by gravity. The fall of the fluid was rapid during the first 2 minutes. By the 4th minute the flow into the muscle slowed sufficiently so that readings could be made. However, the column of saline was not yet in equilibrium with the muscle, for within the next minute a sudden fall of 0.5 to 2.5 mm. occurred, at which later level equilibrium was obtained, except in a few instances (table 1). In the few, a further drop of 2 to 3 mm. water occurred at the end of 10 minutes. The greatest magnitude of error on such occasion was plus 3 mm. water. Inasmuch as 10 mm. water is the allowable error for variations for consecutive readings, the 5 minute period was used which permits constant readings without undue loss of time.

The venous pressure manometer equilibriated with the venous blood in 1 minute. The usual procedure was to start the flow of saline in the intramuscular manometer, then the venous manometer. The venous manometer was read at

TABLE 1

Effect of time on readings of intramuscular pressure

TIME	MANOMETER READINGS, UNCORRECTED FOR CAPILLARITY OR FOR RELATION TO THE NEEDLE IN THE MUSCLE				
minutes					
1	16.5				
2	14.6	14.0	14.0	16.0	14.0
3	13.0	12.0		14.0	
4	12.0		13.0	13.8	13.3
5	11.5	11.5	11.5	13.2	13.0
10	11.5	11.4	11.5	13.2	13.0

various intervals during the 5 minute period, and both at the end of 5 minutes. Respiratory fluctuation in the quiet patient was less than 0.5 cm. water, and in a restless patient up to 1.0 cm. water. An average of 3 or more readings was made of the venous manometer, which included the highest and lowest points of the respiratory fluctuation.

Results. During the administration of 1 unit of plasma (250 cc.), over a period of 90 minutes, intramuscular pressure varied between 70 and 80 mm. water and venous pressure between 6 and 7 cm. water. The widest fluctuations for intramuscular pressure was 10 mm. water and for venous pressure 1 cm. water. All values fell between the limits of error of the method for consecutive readings. Neither the presence of the needle in the muscle, nor the repeated injection of small amounts of saline every 5 minutes for 90 minutes, nor the administration of 1 unit of plasma (250 cc.) altered the initial reading beyond the variations of error of the method.

In figure 1 is shown the effect of the administration of 3 units of plasma on 2 patients who were receiving plasma for other reasons than for the treatment of

shock. A significant increase in intramuscular pressure occurred in the one patient at 45 minutes after the injection was started and 1½ units (375 cc.) of plasma had been administered. Twenty minutes later a significant rise was obtained for the venous pressure. By this time 2 units (500 cc.) of plasma had been given. Both intramuscular and venous pressure remained elevated during the remainder of the one and one-half hours of observation.

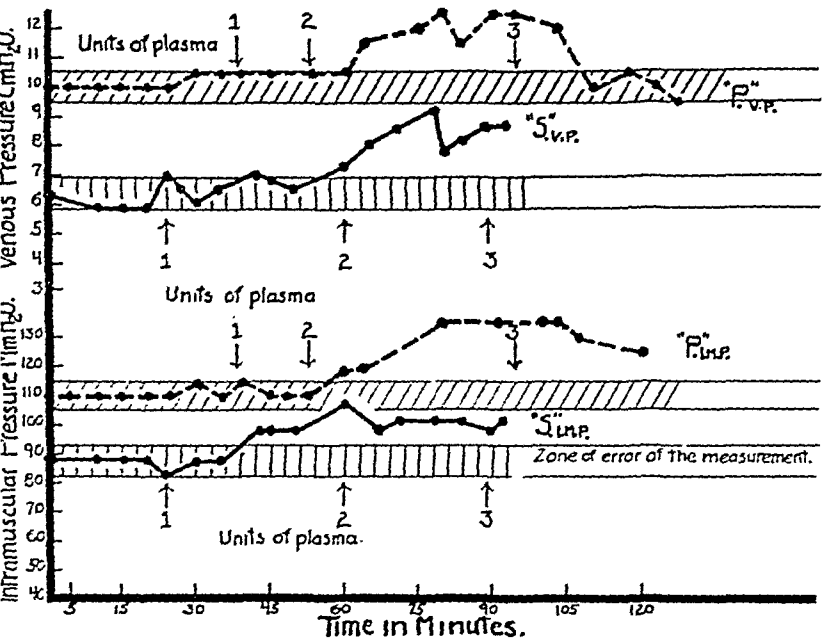

Fig. 1

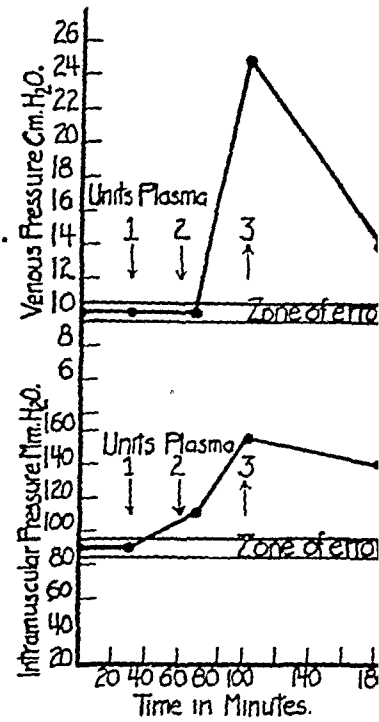

Fig. 2

Fig. 1. Heightening of the venopressor mechanism in the normal person. Plasma was administered for other reasons than for the treatment of shock. Significant increases in intramuscular and venous pressure followed the administration of 1½ and 2 units of plasma. The muscular phenomenon preceded the vascular; and venous pressure varied with the intramuscular pressure.

Fig. 2. Showing the heightening effect of the administration of 3 units (750 cc.) of human plasma on the venopressor mechanism. The rise in intramuscular pressure occurred before the increase in venous pressure. Two units (500 cc.) of plasma were administered before the first significant change took place in intramuscular pressure. The remarkable height of 155 mm. water for intramuscular pressure, and 25 cm. water for venous pressure were obtained after the administration of the 3rd unit of plasma.

The second patient received the plasma more rapidly. He showed an increase in intramuscular pressure 1 hour after the start of the infusion, and after 2 units of plasma had been given. A significant rise in venous pressure occurred 5 minutes after the increase in intramuscular pressure. Both intramuscular and venous pressures remained elevated for the remainder of the 90 minute period of observations. The highest intramuscular pressure recorded (135 mm. water) was observed after 80 minutes when 2½ units (625 cc.) of plasma had been administered.

Two patients suffering from head injuries, semicomatose and without evidence of peripheral circulatory disturbance at the time of admission, were observed during treatment with plasma (fig. 2). The averages of their readings are shown as a single smoothed curve. The first significant increase in intramuscular pressure was observed after 2 units of plasma had been administered, at 70 minutes. The venous pressure was unaltered at 70 minutes. At 100 minutes intramuscular pressure reached the level of 155 mm. water and venous pressure 25 cm. water. Both remained significantly elevated during the remainder of the 180 minute period of observation, although the effect was diminishing. The high levels of intramuscular and venous pressure were similar to the increments observed in our first accidental observation after the administration of 3 units of plasma in the treatment of postoperative depression. This patient's preoperative values of intramuscular and venous pressure were normal.

Figure 3 illustrates the effect of plasma on the failing venopressor mechanism of a patient who had undergone an extensive surgical procedure (thoracoplasty). By the time the operation was completed, a fall of 30 mm. had occurred in the intramuscular pressure. Venous pressure was elevated as a consequence of the inhalational anesthetic (cyclopropane). The administration of plasma was begun immediately after the operation. Venous pressure began to fall during the flow of the first unit, and continued to drop significantly during the administration of the second unit. Both intramuscular pressure and venous pressure rose after the 3rd unit was received. The increase in intramuscular pressure preceded the venous one by 10 minutes. Venous pressure returned to the high level seen immediately after operation. Intramuscular pressure increased from the low postoperative level to above the preoperative one and then stabilized within the zone of the initial reading.

Comments. We reported part of the series of 53 patients (3, 5, 6) studied during operation, in the period in which the development of postoperative depression occurred and to the development of shock, or to recovery. Patients who have been subjected to major surgical procedures showed a decline in intramuscular pressure, which began between 45 and 60 minutes after the procedure was initiated. Clinically they formed 4 groups. These were: 1, the mild postoperative depression with spontaneous recovery within 24 hours; 2, moderate postoperative depression with spontaneous recovery of altered intramuscular and venous pressure values by the 4th day; 3, severe postoperative depression, clinically indistinguishable from shock within the 6th to 12th postoperative hour, and 4, rapidly developing shock, which occurred either during, or immediately after the surgical procedure had been finished. In all but the first group, intramuscular pressure continued to drop after operation to reach the low level of from 20 to 40 mm. water 6 to 12 hours postoperatively (7). (The variations are shown in curves A, A', and B, B', fig. 4.) Venous pressure followed the intramuscular pressure dropping 50 minutes after the first decline in intramuscular pressure. Venous pressure continued to fall and reached the low value of 1 cm. water between the 6th and 12th hour after operation.

When, between 6 and 12 hours after operation, intramuscular and venous

pressure have reached their lowest decline, the clinical condition of the patient in severe postoperative depression is indistinguishable from that of surgical shock which has developed rapidly. In the one, the process was slow and the clinical

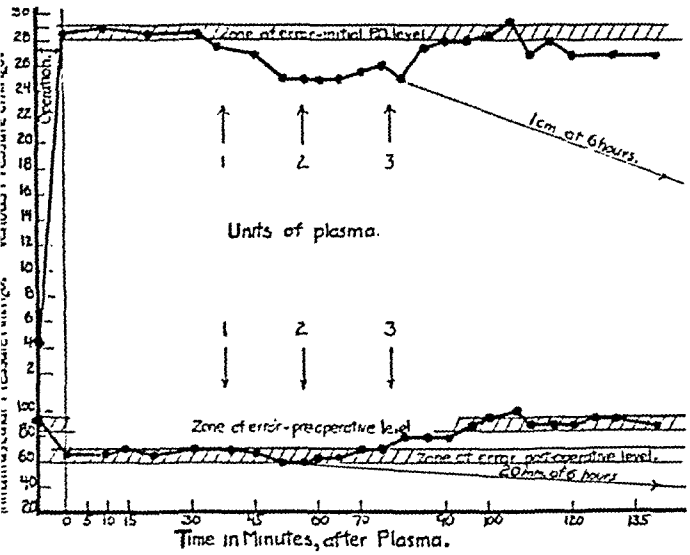

Fig. 3

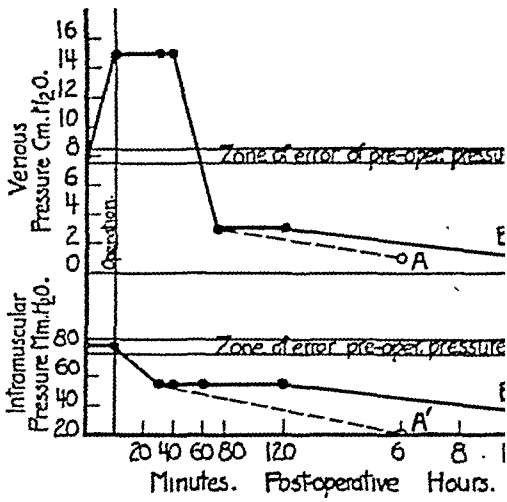

Fig. 4

Fig. 3. Illustrating the restoration of the venopressor mechanism by the use of plasma. Intramuscular pressure dropped 30 mm. water by the end of the operation (thoracoplasty). The increased venous pressure is a consequence of the inhalation anesthesia (cyclopropane), similar to the changes shown in figure 5. During the administration of 2 units of plasma, the trend in venous pressure was significantly downward; intramuscular pressure was unchanged. After the 3rd unit of plasma had been given, the first significant increase occurred in intramuscular pressure. The change in venous pressure followed.

The thin sloping lines (marked 1 cm. at 6 hrs. and 20 mm. at 6 hrs.) represents the usual decline in venous pressure and intramuscular pressure observed in 53 other patients, between the 6th and 12th hour after operation, who were not treated with plasma. (Represented diagramatically in fig. 5.)

In this patient, following the administration of the 3rd unit of plasma, intramuscular pressure returned to its preoperative level. The clinical change for the better was obvious.

Fig. 4. Showing the sequences in the failure of the venopressor mechanism, during and after major surgical procedures.

The points represent averages of data on 8 patients (4 in group A and 4 in group B), who were observed continuously and uninterruptedly, from the time of administration of the anesthetic, to throughout the operation and during the postoperative period.

After inhalation anesthetic, an immediate marked increase occurred in venous pressure. It remained elevated for 50 minutes after intramuscular pressure showed its first significant decline. The drop in intramuscular pressure preceded the one of venous pressure. Both continued to fall, venous pressure to reach 1 cm. water between the 6th to 12th hour, and intramuscular pressure 20 mm. water in group A, and less than 38 mm. water in group B. The clinical picture of failure of the peripheral circulation, which subtly appeared between the 6th and 12th postoperative hour, was indistinguishable from that seen in rapidly developing surgical shock. These patients were not treated with plasma.

picture developed subtly; the vitality of the individual actually disappeared under the eye. In the other, the sequences occurred rapidly. In both instances the clinical evidences of peripheral circulatory failure were present and intramuscular and venous pressures reached their lowest levels. The lowest value

observed in both rapidly developing surgical shock, and in severe postoperative depression was 20 mm. water for intramuscular pressure and 1 cm. water for venous pressure. The only difference in the curve shown in figure 4 in rapidly developing surgical shock is a compression of the time factor. Venous pressure fell within 20 minutes after the initial decline in intramuscular pressure instead of at 50 minutes. The level of 20 mm. water for intramuscular pressure and 1 cm. water for venous pressure was reached in 80 to 120 minutes from the beginning of the operation, rather than in 6 to 12 hours.

We have shown that levels of 20 mm. water for intramuscular pressure during life probably represent an absence of muscle tonus. Intramuscular pressure does not drop any lower immediately after clinical death with the cessation of the heart beat in either the human or in the frog (Rana pipiens) (4).

Figure 4 is an illustration of the failure of the venopressor mechanism as seen in patients who developed severe postoperative depression between the 6th and 12th hours. It is a smoothed curve of continuous, uninterrupted observations on 8 patients. The main points of change only, were marked. Thirty-eight others were also observed, on whom we had continuous, uninterrupted data of one or more of the periods mentioned, but not continuous for the complete sequence from operation to recovery, or death. The data of this group verified the trend shown in figure 4. In 3 patients who developed surgical shock rapidly, the time factor was compressed. The sequences were the same, i.e., intramuscular pressure failed first, and venous pressure 20 minutes later, rather than in the average observed time of 50 minutes. In 4 patients with mild postoperative depression, on whom we had continuous and uninterrupted data from operation to recovery, the opposite holds. The curve flattened, i.e., the average fall for intramuscular pressure was only 20 mm. water as against 40 to 60 mm. water in the severe postoperative group, and for rapidly developing shock. The maximum fall in venous pressure was only 2 cm. water below the preoperative level, as against 6 to 7 cm. water in the severe postoperative depression group, and in rapidly developing shock. In the mild postoperative depression group, recovery of intramuscular and venous pressure occurred within 24 hours, and paralleled the clinical course. The sequences in all groups remained unaltered. Intramuscular pressure dropped during operation, and preceded the fall in venous pressure.

In half of the patients, those receiving inhalational anesthesia, evidence of venoconstrictive compensatory mechanism was observed. There was an immediate elevation of venous pressure which persisted until intramuscular pressure declined. Thereafter, in the usual 50 minute period, venous pressure began to fall, just as it did in those patients who did not show an increased venous pressure after the anesthetic (fig. 4). Thus in the patients who showed an elevation of venous pressure during the operation, the fall in venous pressure from the high compensated level was much greater than the actual 6 to 7 cm. drop from the preoperative value. As much as 10 to 13.5 cm. decline in venous pressure (to a final reading of 1 cm. water) was seen in patients who developed surgical shock immediately after the operation. We believe that the relative increments of loss of intramuscular and venous pressure is a significant feature

in the dynamics of failure of the peripheral circulation. An intramuscular pressure of 20 mm. water indicates a complete absence of muscle tonus and loss of the kinetic energy that tonus could contribute to the venous circulation.

It is conceivable, and likely, in the patient shown in figure 3, that, had the administration of plasma been discontinued before the second unit had been given, both intramuscular and venous pressure would have continued to decline, to reach low levels at the 6th postoperative hour, just as we observed in 53 other patients after operation who had not been treated with plasma.

The marked increase in intramuscular pressure and venous pressure after the administration of 3 units of plasma was observed in 7 patients. No alterations occurred in 1 patient receiving only 1 unit of plasma.

The effect of large quantities of blood plasma on the venopressor mechanism is too startling to be denied. The effect is a slow one, and enabled us to determine the sequences that occurred in the heightening and restoration of the venopressor mechanism. The action of plasma demonstrated that venous pressure fluctuates according to intramuscular pressure. Changes in intramuscular pressure were soon reflected and followed by corresponding alterations in venous pressure.

We consider it especially significant that large quantities (750 cc.) of plasma must be administered to produce this action. Such amounts of plasma with their enormous osmotic effect produced no appreciable rise in venous pressure, until after intramuscular pressure had been significantly increased.

CONCLUSIONS

1. Human plasma in doses of 2 units (500 cc.) significantly increased intramuscular pressure. The administration of 3 units (750 cc.) increased intramuscular pressure to a marked degree.

2. Changes in intramuscular pressure are reflected in corresponding alterations in venous pressure. The failing venopressor mechanism after surgical procedures is marked by an initial decline in intramuscular pressure, to be followed later by a fall in venous pressure. In restoration of the venopressor mechanism through the use of plasma, a rising intramuscular pressure was followed by an increase in venous pressure.

3. The loss or recovery of intramuscular pressure (muscle tonus) appears to be a primary factor that precedes changes in the venopressor mechanism. It precedes either the failure of the venopressor mechanism by its decline during surgical operations, or the recovery of the venopressor mechanism by its increased levels after the administration of plasma.

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THE EFFECT OF INSULIN ON THE RESPONSES OF THE FROG'S HEART AND RECTUS ABDOMINIS TO ACETYLCHOLINE[1]

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Received for publication October 21, 1943

During a study of pituitary influence on the responses to acetylcholine (ACh) of the heart and rectus abdominis muscle of the winter frog (Welsh, unpublished) it appeared possible that the changes produced by hypophysectomy and pituitary gland injection were due to disturbances in carbohydrate metabolism. This belief was strengthened when, in a few preliminary experiments, it was found that the injection of insulin had marked effects on the response of the rectus abdominis muscle, and less marked, but obvious, effects on the heart. The responses were found to be very different depending on the length of time the insulin was allowed to act before the frog was sacrificed. If the insulin was allowed to act for only a few hours at room temperature the subsequent response of the rectus abdominis to a given concentration of ACh was greater than normal, while 24 hours after the injection of insulin the response was subnormal.

During the course of this work Feldberg and Solandt (1942) reported that the excitability to ACh of the longitudinal muscles of the rabbit's intestine is dependent on a supply of glucose. That an adequate source of energy is necessary for a normal response to ACh is not unexpected but it will be indicated later that it is perhaps the rate of utilization of carbohydrate rather than the actual level of glycogen in frog's muscle that determines the degree of response to ACh.

Since the synthesis of ACh, both *in vitro* (Quastel *et al.*, 1936; Mann *et al.*, 1938) and *in vivo* (Kahlson and MacIntosh, 1939; Welsh, 1943), is also normally dependent on the oxidation of glucose it was of interest to learn more of the relation of carbohydrate metabolism to the response of tissues to ACh. To this end a more detailed study was made of the modification of the concentration-response curves for the isolated frog's heart and rectus abdominis following insulin injection in the intact frog. Since determinations of blood sugar and muscle glycogen were also made, knowledge concerning the action of insulin on the frog was extended and results of others were confirmed.

METHODS. The species of frog used was Rana pipiens. These were obtained from a dealer in northern Vermont and freshly collected frogs were received every two weeks. The stock was maintained, unfed, at 15°C. One series of experiments was run at this temperature and a second series at 25°C. The work was done in June and July.

The levels of blood sugar and muscle glycogen were modified by insulin injected in one unit doses into the dorsal lymph sac. At 25°C. only one unit of

[1] Aided by a grant from the Milton Fund of Harvard University.

[2] With the technical assistance of Blanche Jackson and E. E. Boysen.

insulin was administered and frogs were sacrificed 6, 12 or 24 hours afterward. At 15°C., in order to approximate the convulsive stage, a second unit of insulin was injected at the end of 24 hours and allowed to act for 17 to 24 hours longer. The frogs were killed by pithing. Immediately after cannulation of the heart for the Straub preparation, blood samples for sugar determinations were taken directly from the cannula. One gastrocnemius muscle was then quickly isolated for estimating its glycogen content and, in some cases, half of the median rectus abdominis likewise prepared. The other half of the median rectus abdominis and the heart were isolated and set up for determining their responses to ACh.

Blood sugar determinations were made after the method of Miller and Van Slyke (1936). Glycogen was estimated by the method of Good, Kramer and Somogyi (1933) with small modifications as given by Barger and Johnson[3] (1941). The glucose was estimated by the method of Miller and Van Slyke. With each run of glycogen estimations, reagent blanks and a standard solution of pure glycogen were run and the recovery estimated. The average recovery was 99 per cent of the amount added.

The isolated heart was set up in a constant temperature room at 20°C., eserinized, and kymograph records of the inhibition produced by a series of concentrations of ACh were obtained. Each test was exactly one minute in length with a sufficient period of washing between tests for the heart to recover its original amplitude. From the amplitude of beat before the test and that at the end of a one minute application of ACh the per cent inhibition was calculated and used as a measure of effect.

The isolated rectus abdominis muscle, cut to a length of 2 cm. when relaxed, was also eserinized and its contracture in several concentrations of ACh recorded at 20°C. An isotonic lever, with a magnification of 5× and counterweighted with 3 grams, was used to record the contracture. Test periods were 3 minutes in length and a 7 minute period of washing and re-eserinization was allowed between tests. The distance from the base line to the contracture curve at the end of 3 minutes was taken as a measure of effect.

Stock acetylcholine chloride was made up in 5 per cent NaH_2PO_4, sealed in ampoules, heated in a water bath at 100°C for 5 minutes and then stored at a low temperature until used. The stock solution contained one part by weight of the base in 1000 parts of water, and the concentrations as given are by weight of the base.

RESULTS. A. *Fifteen degrees C. series.* The first series of experiments was carried out on animals maintained at 15°C. In this series, glycogen estimations were made only on gastrocnemii. The "normal" responses for rectus abdominis and heart, shown in figure 1 and table 1 are based on averages of eight animals. The mean blood sugar and glycogen values are given in figure 3. Blood sugar values of normal frogs ranged from 49 to 132 mgm./100 cc., with a mean of 80 mgm., and glycogen values from 420 to 1120 mgm./100 grams., with a mean of 784 mgm. The mean blood sugar value is higher than we have since obtained on 18 winter frogs (range, 32–69 mgm./100 cc.; mean, 51 mgm.). The mean

[3] We are indebted to Dr. R. E. Johnson of the Harvard Fatigue Laboratory for guidance in the use of this method.

glycogen value corresponds closely with estimations of Olmsted and Harvey (1927) on gastrocnemii of 3 spring frogs (790, 800, 680 mgm./100 grams). Barger and Johnson (1941) give a long series of values of glycogen content of gastrocnemii of R. pipiens caught in the spring and maintained without food at 4°C for 7 to 10 weeks before use. In one series a mean value of 417 mgm./100 grams was obtained; in another series a mean value of 540 mgm./100 gram. It is to be expected that these values would be lower than those which we obtained for summer frogs which had probably fed within two weeks of the time of use.

Only two frogs were sacrificed 6 hours after one unit of insulin and four frogs after 12 hours; hence the results on these are combined. It may be seen in figure 1 that the average response of the rectus abdominis of these frogs was greater than normal over the entire range of ACh concentrations employed. The maximum contracture occurred at ACh 10^{-5} rather than at ACh 10^{-4} as in the case of the normals. The isolated hearts of these animals were inhibited less than normals at concentrations of ACh of 10^{-8} and higher (table 1). The

TABLE 1

The effect of insulin on responses to ACh (decrease in amplitude) of the frog's heart. 15°C. series

	PER CENT DECREASE IN AMPLITUDE		
	ACh 10^{-8}	ACh 10^{-7}	ACh 10^{-6}
	per cent	*per cent*	*per cent*
Control (8)*	37	61	91
6–12 hrs. after insulin (6)	32	55	81
24 hrs. after insulin (5)	24	42	76
41–48 hrs. after insulin (9)	42	71	96

* Number of frogs used.

blood sugar of these frogs dropped to less than one half the normal value, while the muscle glycogen increased slightly.

Twenty-four hours after one unit of insulin the recti abdominis of five frogs likewise gave contractures greater than normal while the average inhibition of hearts by ACh 10^{-8} and higher was even less than that at 6 to 12 hours. The marked decline in muscle glycogen at this time, while responses of the recti abdominis were still above normal, is to be noted. In an effort to produce a still further decrease in the levels of blood sugar and muscle glycogen six frogs which had already received one unit of insulin 24 hours earlier were given a second injection of one unit and held for a total of 41 to 48 hours after the first injection. These probably closely approached the convulsive stage which Huxley and Fulton (1924) found to occur 60–70 hours after a single injection of 0.45 to 3 units of insulin when frogs were maintained at 15°C. In contrast to the other insulin-injected frogs the responses of the recti abdominis were below normal while the isolated hearts showed greater than normal inhibition by ACh.

B. *Twenty-five degrees C. series.* Since the action of insulin in producing convulsions in the frog is greatly accelerated with an increase of temperature (Hux-

ley and Fulton, 1924; Olmsted, 1924; Barlow, Vigor and Peck, 1931) a second series of experiments was performed on frogs at 25°C. In addition to the estimations of the glycogen content of gastrocnemii, estimations were made of glycogen in one half of the median rectus abdominis of some frogs. The relative change of glycogen level produced by insulin was essentially the same in both muscles.

Ten control frogs were placed at 25°C and allowed to remain for at least 24 hours. The average concentration-response curve for their isolated rectus abdominis muscles was almost identical with that of the 15° series (cf. figs. 1

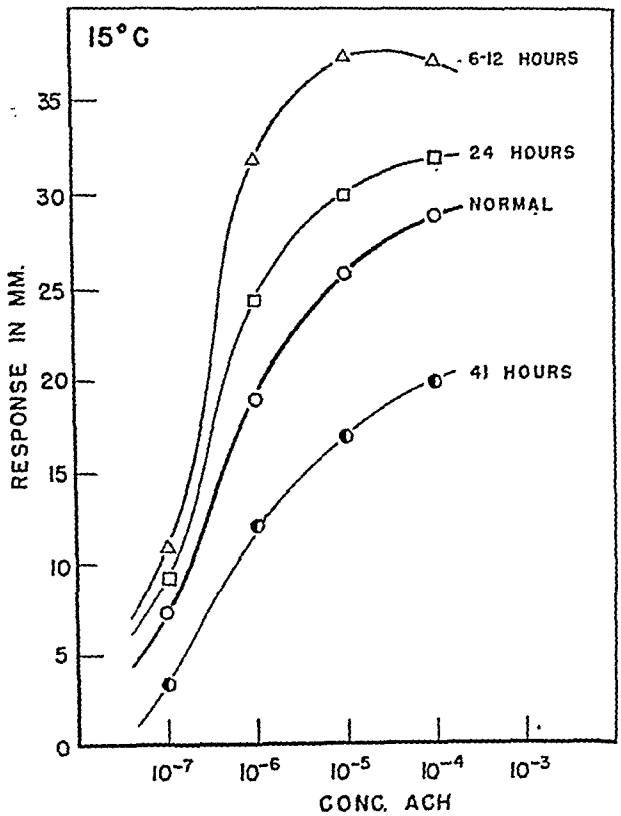

Fig. 1

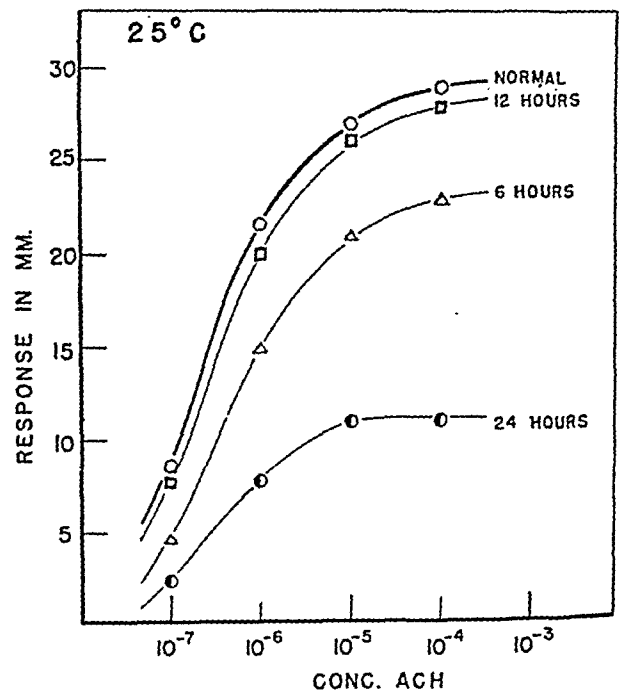

Fig. 2

Fig. 1. Concentration-response curves for isolated recti abdominis of normal frogs and of frogs previously injected with insulin. Frogs maintained at 15°C. Tests made at 20°C.
Fig. 2. As in figure 1, but frogs maintained at 25°C

and 2). The response at ACh 10^{-4}, as measured by the lever rise after 3 minutes, was the same (29 mm.) in both series. Mean blood sugar and muscle glycogen (gastrocnemius) values are shown in figure 4.

Nine frogs, adapted to 25° for at least 24 hours, received one unit of insulin in the dorsal lymph sac and were sacrificed at the end of six hours. Blood sugar values had already dropped remarkably while the average glycogen content of the gastrocnemii had increased to 680 mgm./100 grams (fig. 4). In spite of the increase in muscle glycogen the responses of the subsequently isolated muscles were below normal over the entire range of ACh concentrations employed (fig. 2).

Eight frogs were adapted to 25°C. for 24 hours or longer, injected with one unit of insulin and sacrificed after 12 hours. Blood sugars of this group were also low and glycogen values of gastrocnemius and rectus abdominis muscles now showed a decline. The averaged concentration-response curve for the recti abdominis of this group, however, was nearer that of the controls than in the case of the 6-hour group. This is without satisfactory explanation but there is one suggestion which may be offered. As a matter of convenience all frogs in the 6-hour group were injected with insulin in the morning (8:15 to 9:30 a.m.) and killed in the afternoon, while frogs in the 12-hour group were injected with insulin in the evening (7:30 to 11:00 p.m.) and killed the following morning. It is possible that a 24-hour cycle in the metabolism of the frog results in the response to insulin differing with the time of injection. Agren, Wilander and

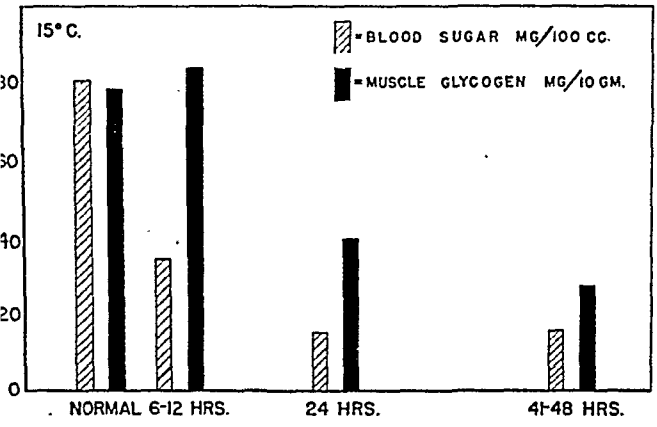

Fig. 3

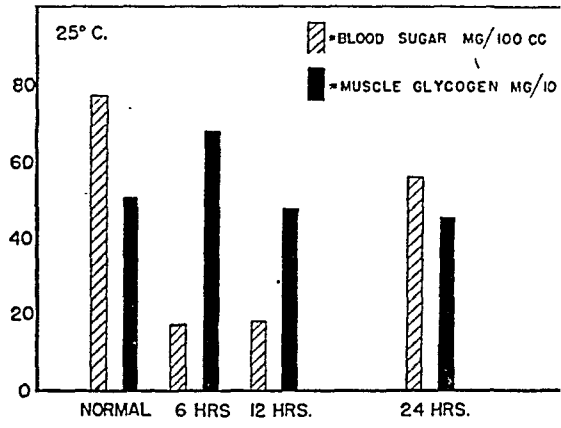

Fig. 4

Fig. 3. Average blood sugar and muscle (gastrocnemius) glycogen levels of a group of normal frogs and of groups of frogs at intervals after injection with insulin. Frogs maintained at 15°C.

Fig. 4. As in figure 3, but frogs maintained at 25°C.

Jorpes (1931) found that it took twice as much insulin to produce convulsions at night in mice as was required during the day.

Nine frogs adapted to 25°C were each administered one unit of insulin and tests were made 24 hours later. The average blood sugar level had increased markedly over the two earlier insulin groups. Glycogen levels of gastrocnemii had returned to within the range of the untreated controls. In spite of the return toward normal levels of blood sugar and muscle glycogen, the response to ACh was far below normal (fig. 2). This was the most striking demonstration that the response of the isolated rectus abdominis to ACh is not directly related to the level of muscle glycogen.

It was not possible to test all of the hearts of the insulin treated frogs at 25°C., but at concentrations of ACh (10^{-8}, 10^{-7} and 10^{-6}) where the amount of inhibition was between 29 and 79 per cent the responses bore a consistent relation to the time of action of the insulin. This may be seen in table 2. With one exception—the average response at ACh 10^{-6}, 6 hours after insulin—the hearts

of insulin treated frogs were progressively "less resistant" to ACh (i.e., showed greater decrease in amplitude of beat) with the lapse of time after the injection of insulin. Thus, in this series, while the ACh contractures of rectus abdominis muscles of insulin treated frogs were all less than normal, the per cent decrease in amplitude of beat of isolated hearts was greater than normal.

DISCUSSION. It is clear from these studies that there is a marked modification in the response to ACh of the frog's heart and rectus abdominis produced by the administration of insulin. Depending on the time after injection and on the temperature at which the frogs are maintained, the contracture of the rectus abdominis produced by ACh may be greater than normal, or less than normal; while the per cent decrease in amplitude of heart beat produced by ACh bears an inverse relation to the response of the rectus abdominis. This difference in response is as one might expect since skeletal muscle of the frog responds to ACh by contracting, while the heart shows a negative inotropic response to ACh.

TABLE 2

The effect of insulin on responses to ACh (decrease in amplitude) of the frog's heart. 25°C. series

	PER CENT DECREASE IN AMPLITUDE		
	ACh 10^{-5}	ACh 10^{-7}	ACh 10^{-8}
	per cent	*per cent*	*per cent*
Controls (6)*	29	46	68
6 hrs. after insulin (5)	33	53	65
12 hrs. after insulin (4)	35	55	78
24 hrs. after insulin (5)	38	60	79

* Number of frogs used.

That insulin produces its effects by a modification in carbohydrate metabolism seems most probable, but the exact nature of the changes responsible for the varied responses to ACh is not clear. The blood sugar level of the frog would appear to be of little direct importance because the testing of hearts and muscles was done after isolation, and over a period of 2 to 5 hours after removal from any possible influence of changing levels of available glucose. Since it was thought that the response to ACh might show a direct relation to the level of muscle glycogen the estimations of glycogen were made. Measurements of cardiac muscle glycogen could not have been made until after testing the hearts, hence none were done. More estimations of glycogen would have been made on rectus abdominis muscles if the amounts of tissue had been sufficient for accurate measurements. Since one half of the muscle was needed for the ACh tests this left usually less than 150 mgm. for the glycogen determination. If the changes in the level of glycogen of the gastrocnemius muscles may be relied upon to give an indication of the changes in the rectus abdominis muscles there appears to be no constant relation between glycogen level and response to ACh. In the 15°C. series there was a small average increase in glycogen of the gastroc-

nemii, 6 to 12 hours after insulin, and a large increase in the response to ACh of the rectus abdominis muscles. In the 25°C. series a marked increase in glycogen of the gastrocnemii was found 6 hours after insulin but the response of the rectus abdominis muscles to ACh was below normal. Twenty-four hours after insulin (at 25°C.) the glycogen level of gastrocnemii was only slightly below normal and that of recti abdominis showed an increase over the level at 12 hours, yet the contractures produced by ACh were greatly subnormal. It seems doubtful, therefore, that the glycogen level, *per se*, determines the degree of response of the rectus abdominis to ACh. Earlier investigations of the ability of frog's muscle to respond to electrical stimulation under both aerobic and anaerobic conditions have shown little or no relation between glycogen levels and the ability to do work (see Barger and Johnson, 1941, for references).

If one assumes (from evidence of Krebs and Eggleston, 1938) that extra insulin produces an increased oxidation of carbohydrate by the skeletal and cardiac muscle of the frog one then has a possible explanation for the observed phenomena. In the series at 15°C., the increased responses of the muscle and the decreased inhibition of the heart at intervals up to 24 hours after insulin might then be explained as the result of a greater than normal rate of utilization of carbohydrate. Failure to find a supernormal phase in the 25°C. series could be explained by the much greater rate at which insulin acts in the frog at 25°C. as compared with 15°C. (Olmsted, 1924). The subnormal responses of the rectus abdominis muscles and the greater than normal inhibition of hearts would result from a lower than normal rate of utilization of carbohydrate. A more detailed analysis of the effect of insulin on the response of muscle to the neurohumor, acetylcholine, must await further studies of the precise mechanism of insulin action such as those of Krebs and Eggleston (1938), Rice and Evans (1943) and Sacks (1943). However, the results from this study provide evidence for a relationship between carbohydrate metabolism and the response to ACh of frog's skeletal and cardiac muscle and this, coupled with the demonstrated importance of glucose oxidation in ACh synthesis, makes it apparent that endocrine, or other, disturbances in sugar metabolism might have far reaching effects on cholinergic systems.

SUMMARY

The responses of the isolated frog's heart and rectus abdominis muscle to acetylcholine (ACh) may be modified by previous administration of insulin to the intact frog. At a temperature (15°C.) at which the action of insulin on the frog is relatively slow, its effect, during the first 24 hours, is to increase the responsiveness of the rectus abdominis to ACh and to decrease the per cent inhibition of the heart at concentrations of 10^{-8} and higher. At 25°C. the responses of the rectus abdominis to ACh, at intervals of 6 to 24 hours after insulin, are subnormal, and the per cent inhibition of the heart greater than normal.

These modifications in response are shown not to be directly related to blood sugar levels nor to levels of muscle glycogen but it is suggested that they may be due to changes in the rate of utilization of carbohydrate.

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FUNCTIONAL ALTERATIONS IN MOTOR AND SUPRANUCLEAR MECHANISMS IN EXPERIMENTAL CONCUSSION[1]

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Received for publication November 29, 1943

Denny-Brown and Russell (1941) have considered in some detail the functional alterations produced by experimental concussion. They struck the movable head of the cat with a blunt metal knob attached to the end of a rigid pendulum. Their observations on respiration, blood pressure and certain somatic reflex activities led them to conclude that moderate concussion is a paralysis of reflex activation of motor centers in the brain while the centers themselves may even be stimulated. They also concluded that severe concussion produces complete paralysis from which recovery is gradual. Sometimes, despite artificial respiration, death occurred and was thought to be due to rapid circulatory collapse similar to that of primary shock.

In the work of Denny-Brown and Russell (1941) a certain velocity of the striking mass was thought to be required to produce the concussion which they designated "acceleration concussion." They stressed the importance of allowing the head to move when struck. On the other hand Duret (1920), Scott (1940) and Gurdjian and Webster (1943) produced concussion by striking the fixed head.

Denny-Brown and Russell (1941) recognized a second type of experimental concussion which they named "compression concussion." Injury was produced by rapid and momentary introduction of air into the cranium in order to cause a sharp rise of intracranial pressure. Slow and prolonged increases in pressure caused other damage but not concussion. "Compression concussion" differed from "acceleration concussion." In the former the initial blood pressure rise was not so great and was less steep, and the respiratory center was more easily and severely affected; the pinna reflex returned in the former before the corneal reflex.

In order to relate the immediate effects of experimental concussion upon brainstem reflex functions to the effects upon the electrical activity of the cerebral hemispheres, Williams and Denny-Brown (1941) obtained continuous electroencephalographic records from cats concussed by a hammer blow on the movable skull. A diminution in electrical activity of both hemispheres occurred immediately upon production of concussion. Local contusion caused changes in electrical activity of the contused areas only, unless concussion resulted from the blow. In studying the electroencephalogram in acute head injuries of humans,

[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Northwestern University.

[2] The authors wish to thank Mr. W. A. McKinley, Dr. Abraham Wikler, Miss Elizabeth Cowgill and Mr. W. A. Rambach for assistance during various phases of the study.

Williams (1941) found that the characteristic slow waves of the acute stages were invariably generalized. He pointed out that this supports the view that cerebral concussion is the result of a widespread disorganization of cerebral function.

It is evident that concussion is characterized by profound physiologic disturbances which set in immediately. It is also clear that the parts of the neuron disabled, the ultimate nature of the disability, its distribution and the types of gross insult which will mediate this disability of concussion are open questions.

Our work was undertaken primarily to determine the functional state of motor parts of the brain in experimental concussion. In addition it has afforded an opportunity to relate external manifestations of concussion to internal functional changes and to compare concussion produced by different methods.

METHODS. Cats weighing 2½ to 3 kgm. were used. Their regular diet, which included yeast and cod liver oil, was supplemented with calcium phosphate to insure maximum bone strength. All experiments were acute. The animals were lightly anesthetized with chloralosane in all experiments, an area of the skull was exposed and through a drill hole or a trephine opening fine bipolar electrodes were oriented in the brain with the Horsley-Clarke instrument. Electrodes were cemented in place in a metal collar attached to the skull by screws. Often two regions were prepared for stimulation in one animal. When the cement solidified the Horsley-Clarke instrument was removed and leads from a 60 cycle A.C. stimulator, having an output of 0 to 50 volts,[3] were securely attached to the electrodes. Preceding concussion three or more control values for the thresholds of the responses under test were recorded at about 5-minute intervals. At each period of recording, the corneal reflex and often the pupillary-light, startle and pinna reflexes were tested. The animal was concussed as quickly as possible after the last control test period. Reflexes were tested immediately and effect on respiration was observed, as were other alterations. Threshold values for the responses to stimulation were obtained 1 to 30 seconds after the onset of concussion. Thresholds and reflexes were followed until the termination of the experiment. Often an animal was concussed more than once.

For the most part concussion was produced by striking an exposed portion of the movable skull, usually the temporo-parietal region. The striking implement was a pendulum-like apparatus similar to that described by Denny-Brown and Russell (1941).

A smaller series of cats was concussed by a different method, the essential feature of which was transmission of force through a fluid column to the intracranial space. The apparatus was modeled after that of Clark (1943) but possessed a snugly fitting piston and a valve in the cannula which added greatly to the ease and controlability of concussion production. A metal cannula with two inlets, one of them bearing a needle valve, was filled with physiologic saline solution and screwed tightly into a 6 mm. trephine hole in the skull. The dura was usually removed to the edges of the trephine hole. Copper tubing leading from the bottom of an upright cylindrical metal chamber was fastened to the other inlet of the cannula. Tubing and cylinder were filled with saline through the

[3] The voltages recorded were root-mean-square voltages.

valved opening of the cannula and a snugly fitting plunger was inserted into the open upper end of the cylinder. Concussion was produced by dropping a 200 gram disc from a height of 2 m. onto the plunger which was allowed to drive into the cylinder displacing 1.6 ml. of saline. As an essential part of the concussive procedure the needle valve in the cannula was opened variable degrees to produce concussions of variable intensities.

Other cats were used to test the effect of striking the exposed immobilized skull with the pendulum. One exposed temporal region was fitted firmly against a thick metal plate screwed to an unyielding wooden supporting assembly. The opposite exposed temporo-parietal region was struck with the pendulum as in the experiments with the movable head. Care was taken to ascertain that the metal contacts with the skull were surface, not edge, contacts.

At the termination of an experiment the animal was perfused with 10 per cent formalin and the brain and upper cervical spinal cord removed. Subsequently, the sites of stimulation were verified either by gross or by microscopic examination.

RESULTS. (*1*) *Concussion produced by pendulum blow upon movable head.* Fifty-five concussions were produced in 28 cats. In 7 of these and 9 other animals mainly in the earlier part of the work, 34 other attempts to produce concussion were unsuccessful. The points stimulated and the number of concussions in which each was under observation were as follows: hypothalamus, 1; hypothalamus and motor cortex in same preparation, 1; oculomotor nucleus, 10; trigeminal motor nucleus, 1; facial nucleus, 18; inspiratory center, 7; facial nucleus and zygomatic branch of the facial nerve, related to each other in the same preparation, 9; facial nucleus and nerve bundles in the pons between the nucleus and the genu of the facial nerve, related to each other in the same preparation, 1; facial nucleus and the face area of the motor cortex, related to each other in the same preparation, 2; forelimb area of the motor cortex and forelimb fibers in the basis pedunculi, related to each other in the same preparation, 5.

Grossly observable signs of concussion. Observations were generally begun not longer than 2 seconds after production of concussion. Immediately and simultaneously respiration ceased, the canthus (wink) and pupillary-light reflexes vanished, the nictitating membrane retracted, the pupils dilated and startle and pinna reflexes disappeared. These activities returned to normal approximately in the order in which they are listed. The canthus reflex usually returned in 10 to 150 seconds; the pinna reflex, in 2 to 15 minutes. In lighter concussions pupillary dilatation and nictitating membrane withdrawal were not instantaneous, nor were they as extensive as in more severe concussions. Respiration seemed to be arrested between inspiration and expiration. It usually returned spontaneously in 10 to 120 seconds. In 7 instances of very severe concussion respiration did not return spontaneously. Manual artificial respiration was successful in 4 of these.

The intensity of the concussion was proportional to recovery times of the observable signs mentioned above, especially of the canthus reflex and, to a somewhat lesser degree, of respiration.

Immediate effect of concussion upon thresholds for elicitation of responses by central stimulation. The thresholds for supranuclear motor system responses (motor cortex, hypothalamus and tegmentum) rose sharply upon concussion in 66 of 80 observations. In just 2 instances, both involving a pilomotor response to hypothalamic stimulation, was a decrease in threshold of a supranuclear response noted. It seemed likely in most of the remaining 12 observations that failure to detect a rise in thresholds was due to concentration of attention on other responses.

TABLE 1

Concussion effects in cell groups and in fibers related in same concussions

CAT NO.	THRESHOLD*		SITE OF STIMULATION	RESPONSE
	Fibers	Cell groups		
49	2.5	3.5	Basis pedunculi; motor cortex	Forelimb movement
	2.5	7.5		
50	4.5	3.5		
	5.5†	7.0		
50	5.0	5.0		
	5.0	>20.0		
52	1.0	0.8	Facial nerve fibers between nucleus and genu; facial nucleus	Lid closure
	1.0	2.5		
41	0.9	1.0	Zygomatic branch of facial nerve, facial nucleus	Lid closure
	0.9	1.2		
44	0.6	0.7		
	0.6	1.8		
45	0.6	0.8		
	0.6	1.8		

* Thresholds in volts. The upper number of each pair is the control value; the lower number, the concussional value.

† This is the one instance of slight rise mentioned in the text. At this level of stimulation, 0.5 volt was the smallest increment used in testing. Control values in this animal were fluctuating between 4.0 and 5.0 volts.

Quite different were the results obtained from stimulation in cranial motor nuclei during concussion. Of 45 such responses observed, 19 showed no change of threshold upon concussion, 6 showed slight but sharp decrease in threshold and 20 were characterized by a sharp rise.

Data on the response of inspiration obtained by stimulating the reticular formation of the medulla oblongata are not included in the above surveys. From 7 observations of this response in concussion it was found that the threshold remained unchanged in 3, sharply decreased in 2 and sharply increased in 2.

The thresholds for responses from cranial motor nuclei and from the respiratory

center generally were elevated little or not at all upon concussion, although reflex activation of these nuclei and spontaneous respiration were abolished.

In 9 instances stimuli were applied to nerve fibers which coursed through the brain (descending motor tracts or cranial motor nerve fibers). Concussion did not alter thresholds for elicitation of responses in 7 experiments, lowered the threshold upon one occasion and evoked a slight rise in the remaining instance. On 9 occasions the zygomatic branch of the facial nerve was tested during concussion. It was not affected.

Among the above mentioned experiments were some in which electrodes were placed in pairs in the same preparation, one electrode stimulating a cell group and the other stimulating axons derived from the cell group. Following concussion the threshold of the fibers was determined first, the threshold of the cell groups next (table 1). As indicated above, thresholds of the cell group were elevated in concussion while those of fibers were not.

Threshold values. Control thresholds of the motor cortex were distributed between 3.0 and 5.0 volts. The concussion increments ranged from 1.5 to more than 17 volts. Control thresholds of other supranuclear responses ranged from 0.8 to 3.0 volts, with concussion increments ranging from less than 1.0 to 7.0 volts. Increments of supranuclear responses were generally greater than those of other responses.

In the cranial motor nuclei, control thresholds were 0.7 to 1.6 volts. The concussion increments ranged from less than 1.0 to 2.5 volts. The highest absolute threshold value recorded was 3.5 volts except in one instance of fatal concussion. Thresholds in the inspiratory center behaved much like those in the cranial motor nuclei except that the control values were as much as 2.0 volts.

Control thresholds of nerve fibers ranged from 0.5 to 1.6 volts except in the basis pedunculi. There they were higher, as can be seen in table 1.

In the two instances in which thresholds of supranuclear responses decreased upon concussion, values were lowered 1.0 and 1.2 volts. In the motor nuclei they were lowered 0.2 volt; in the inspiratory center, 0.4 volt and in nerve fibers, 0.4 and 0.2 volts.

Recovery of thresholds. Threshold increase and threshold recovery time were roughly proportional to intensity of the concussion. Unlike threshold rise, threshold recovery was relatively gradual. Recovery curves usually were steep at first and then tended to level off as the values approached the control values. In a number of instances thresholds returned to normal in a fraction of a minute to 5 minutes following the acute concussional rise. In some experiments in which electrodes were in the facial nucleus or in the inspiratory center complete recovery of thresholds coincided with return of the canthus reflex or of spontaneous respiration. In one instance the threshold of the motor cortex returned with the canthus reflex and spontaneous respiration at one minute. As a rule return to normal of the grossly observable signs of concussion was unaccompanied by a corresponding recovery of the thresholds under test. Nearly half of the thresholds did recover completely in variable periods up to 1½ hours. The rest recovered only partially or not at all in the periods allowed which were 20 minutes

to 2 hours. Protracted or incomplete recovery was not due to skull fracture or to gross hemorrhage.

Relation of respiratory failure to concussional changes. In order to eliminate anoxia as a causative factor of acute concussional threshold changes the trachea was clamped in 5 cats which had recovered from concussion. The pupils began to dilate only after 1 to 2 minutes, the canthus reflex disappeared in 2 to 3 minutes and thresholds did not begin a gradual rise until 2½ to 5 minutes. Five other concussed animals exhibited effects of anoxia superimposed upon the early recovery period.

Fatal concussion. In one cat with the electrodes in the facial nucleus the threshold rose to 20 volts from a control value of 0.8 volt. After 90 seconds, when the threshold had fallen to 14 volts, an irreversible rise began. Respiration ceased upon concussion and manual artificial respiration was unsuccessful. The canthus reflex disappeared at concussion and never returned. Agonal piloerection set in 120 seconds after the blow. Death seemed to be due to circulatory collapse. This was no doubt as severe a concussion as could be produced with the pendulum striking the movable head, demonstrating that in concussion of unusual severity the threshold of even a cranial motor nucleus can be greatly elevated.

In two experiments in which thresholds did not rise unusually high immediately upon production of severe concussion, artificial respiration was not applied early enough to prevent death. In a third it was not applied at all. As anoxia set in thresholds rose irreversibly and death occurred from respiratory failure.

(2) *Concussion produced by transmission of force through fluid.* Two series of animals were used in these experiments. In the first series the needle valve was not opened; the fluid was driven into the cranium and allowed to escape within 2 seconds after the blow. Five concussions were produced in 4 animals with electrodes in the inspiratory center, one in a cat with electrodes in the inspiratory center and in the motor cortex and one in an animal with electrodes in the hypothalamus. One of these was of the immediately fatal type described above in which death was probably due to circulatory collapse. Four of the remaining 6 were more severe than any we encountered except the 2 which resulted in immediate fatality of the circulatory failure type. Nevertheless all thresholds, i.e., those for supranuclear responses, inspiration and tongue movement in response to stimulation of hypoglossal fibers in the medulla oblongata, changed in complete accordance with the pattern reported for pendulum concussion.

In the second series the needle valve was opened varying degrees before the blow, permitting fluid to escape through an opening of adjustable size at the moment of the blow. This method provided the greatest uniformity of results. Table 2 contains a summary of data from all concussions produced by it. Results conformed in all respects with those already recorded. Thresholds of the supranuclear responses (motor cortex and hypothalamus) showed greater rise and slower recovery than those of cranial motor nuclei (facial nucleus). The inspiratory center behaved much like a cranial motor nucleus. Thresholds of motor nerve fibers (hypoglossal fibers) were not elevated. Although not indi-

cated in table 2, pupillary dilatation and nictitating membrane withdrawal occurred as in the pendulum experiments. In addition conditions were favorable for the observation of the slight but constant startle which occurred at the moment of a blow.

TABLE 2

Results of concussion produced with hydraulic system

CAT NO.	CONCUSSION NO.	VALVE OPENING	RETURN OF:			SITE OF STIMULATION	RESPONSE	STIMULATION THRESHOLDS		
			Canthus reflex	Respiration	Pinna reflex			Control	Concussional	Recovery
		turns	*seconds*	*seconds*	*seconds*			*volts*	*volts*	*seconds*
53	1	1	30	15	120	Facial nucleus	Lid closure	0.7	0.8	45
	2	¾	45	30	120			0.7	1.4	45
	3	½	60	50	135			0.7	5.0*	100
54	1	1	30	8		Motor cortex	Lid closure	2.5	3.5	45
	2	¾	85	25				3.0	14.0	105
	3	½	150	60				3.0	>20.0	600
55	1	¾†	20	15		Hypothalamus	Piloerection	1.1	2.0	45
	2	½	30	15				1.2	1.8	1500
	3	¼	900	120‡				1.3	>10.0	§
56	1	1	180	60		Hypothalamus	Piloerection	2.0	>10.0	§
57	1	1	5	¶		Reticular formation of medulla	Inspiration	1.8	1.4	
	2	¾	60	¶				1.8	3.0	§
	3	½	270	¶				2.5	2.5	
57	1		Same concussions			Reticular formation of medulla‖	Tongue movement	1.6	1.0	
	2							1.6	0.8	
	3							1.6	1.0	

* This concussion was one of the most severe in which electrodes were in a cranial motor nucleus.

† One turn did not produce concussion.

‡ Artificial respiration applied at 60 seconds.

§ Recovery incomplete in time allowed.

¶ Respiration ceased but elicitation of inspiratory response interfered with observation of exact time of recovery of respiration.

‖ Electrodes were on hypoglossal fibers.

The cannula was screwed into the parietal bone except in cat 56 in which it was placed in the squamous portion of the occipital bone. Cats 55 and 56 were prepared and concussed together. From table 2 it is evident that force applied to the brain caudal to the tentorium cerebelli was more effective in producing concussion than force applied rostral to it.

(3) *Concussion produced striking the immovable head with the pendulum.* Whenever the pendulum was used magnitude of the blow was measured in terms of the

angle between the shaft of the pendulum in its displaced position preparatory to striking a blow and the shaft in its mean position. The blow was recorded as a fraction on the basis of a 180° displacement as unity; e.g., a blow delivered with the pendulum shaft poised at the start 90° from its mean position was recorded as a one-half blow.

Four cats were used. The first was given a three-quarter blow. This was the lightest blow used to produce concussion in the movable head. The striking end of the pendulum crashed through the fixed head, demolishing it, and struck the metal support plate.

The second cat was first struck with a one-eighth blow of the pendulum. It had no effect. A one-quarter blow sprang the right tempero-parietal suture slightly and produced a very severe concussion. The pupils dilated widely. Artificial respiration was begun at 90 seconds. Spontaneous respiration began at 135 seconds. The canthus reflex returned in 285 seconds. About one hour later this same cat, but with the head movable, was struck a one-quarter blow on the left temporo-parietal region. There was no effect. A one-half blow struck in the same manner produced a very slight concussion with the canthus reflex and respiration both returning in less than 10 seconds.

An initial one-sixth blow of the pendulum against the immovable head resulted in a moderate concussion in the third cat. Respiration ceased for 15 seconds and the canthus reflex was inert for 50 seconds. A second one-sixth blow produced a very severe concussion. Artificial respiration was begun at 135 seconds. Respiration became spontaneous at 255 seconds. The canthus reflex did not return in the 10-minute recovery period allowed. A one-half blow of the pendulum was then delivered against the animal's head. The blow crushed the skull from both sides and part of the cerebrum herniated.

In the fourth cat 6 drill holes 1 mm. in diameter were made in the parietal and occipital bones on the left side of the skull and 4 holes on the right side. Several punctures were made in the subjacent dura at each hole. A one-sixth blow of the pendulum failed to impart concussion. A one-fourth blow produced a severe concussion. The right temporo-parietal suture sprang slightly. Respiration returned at 65 seconds, the canthus reflex at 190 seconds.

A slight startle was observed at the moment of the blow in each concussion of the fixed head.

Discussion. By stimulating motor portions of the brain we were able to collect data which we believe constitutes the first direct demonstration of localized internal functional alterations in brain concussion. Fascicles of nerve fibers did not exhibit impaired excitability during concussion. The excitability of cell groups comprising cranial motor nuclei was impaired less frequently, to a smaller degree and for shorter intervals than was that of such supranuclear motor regions as the brain-stem tegmentum, hypothalamus and motor cortex. Excitability of the inspiratory center in the reticular formation of the medulla oblongata differed from that of other supranuclear systems tested and was much like that of a cranial motor nucleus in concussion.

Differential functional alterations in the brain during concussion were found

in analysing the behavior of pupillary-light and canthus reflexes in relation to threshold changes in the oculomotor nucleus and the facial nucleus. In a majority of instances these thresholds rose only slightly or not at all while, concomitantly, the reflexes were unelicitable. Here again the inspiratory response resembled a cranial motor nucleus response. In half the observations on the inspiratory center in concussion we found that thresholds for the inspiratory response did not rise although spontaneous respiration was abolished.

A stimulation of certain neural components in concussion may have taken place. A slight transient startle response usually occurred at the instant of the blow. The tongue muscles often contracted tonically when the blow was struck and the orbicularis oculi muscles sometimes reacted in the same way. These contractions endured for periods up to about 60 seconds. Dilatation of the pupil and withdrawal of the nictitating membrane may be mentioned here, although the former could have been a manifestation of oculomotor paralysis. In addition, Denny-Brown and Russell (1941) noted inspiratory gasp. There is good correlation between the visible signs of central stimulation-like phenomena and the lowered thresholds to central stimulation sometimes observed in concussion. It is reasonable to suppose that these two features had a common basis.

We found some evidence that thresholds, principally of supranuclear responses, underwent slight concussional elevations even though the canthus reflex was unaffected or was but momentarily extinguished. These observations were not numerous and were not included among results because we chose abolition of the canthus reflex as the criterion of concussion. Infrequently respiration ceased for 10 or 15 seconds while the canthus reflex remained intact. The converse of this was found occasionally. It would be justifiable to say that very light concussions may occur without extinction of the canthus reflex.

Occasionally we encountered subdural hemorrhages, punctate hemorrhages in the brain substance and, rarely, fractures of the skull. Results in these experiments did not differ from those in which such complications were absent.

No basis was found for distinguishing qualitatively a concussion produced by one method from one produced by another. We believe that concussion results simply from the application to the brain of an adequate force. A consideration of the following formula will elucidate this statement:

$$F = \frac{m(v_1 - v_2)}{t}$$

In the pendulum experiments F = force applied to the skull; m = effective mass of the striking end of the pendulum; v_1 = velocity of the pendulum when it contacts the skull; v_2 = velocity of the pendulum when it ceases to act upon the skull; t = time during which velocity of the pendulum changes from v_1 to v_2.

A three-quarter swing, which produced concussion in the movable head (example A), demolished the fixed head (example B) because the force applied to the head was much greater in B than in A. The values of m and v_1 were the same in A and B and the value of t was probably smaller in B than in A. In

example A the pendulum struck the skull, continued its swing briefly in contact with the skull and was halted by a stop interposed in the path of its rigid shaft while the head continued to move and was halted slowly by a heavily padded stop. The value of v_2, which was the minimum velocity of the pendulum while it was still in contact with the head, was only slightly less than v_1; most of the force of the pendulum was spent against its stop. In example B the pendulum struck the skull and was stopped by it: $v_2 = 0$. Therefore the quantity (v_1-v_2) was much greater in B than in A; and F_B (force in example B) was much greater than F_A (force in example A). To produce a concussion in the immovable head like that in the movable head (example A), the force applied must be made to equal F_A. It will be necessary either to reduce the quantity (v_1-v_2) by reducing v_1, to reduce m or to increase t. Experimentally we reduced v_1 by using a shorter swing of the pendulum. By this means we produced concussion in the immovable head comparable with that in the movable head (example A). In the course of our experiments with the movable head we varied m by adding weight to the pendulum shaft. With no weight added a three-quarter swing did not produce concussion. With sufficient weight added a three-quarter swing did produce concussion.

It must be remembered that when the skull was struck not all of the force was transmitted to the brain. It is possible that the portion of the force transmitted to the brain, or to parts of the brain most concerned in concussion, varied with the region of the skull struck. We found it easier to generate concussion by striking the temporal region than the anterior inter-parietal region of the skull.

In the hydraulic method of producing concussion F = force applied to the plunger; m = mass of the disc falling on the plunger; v_1 = velocity of the disc when it contacts the plunger; v_2 = velocity of the disc when it ceases to act upon the plunger; t = time during which velocity of the disc changes from v_1 to v_2.

We found it most practical to apply a constant force to the fluid column and to allow part of it to be dissipated through the needle valve in the cannula. The force (total pressure) transmitted to the brain could thus be controlled. The valve opening also functioned to prevent displaced fluid from being locked in the cranium. We found that force applied caudal to the tentorium cerebelli was more effectively transmitted to the brain-stem structures involved in concussion than force applied rostral to it, but because of the greater convenience the latter procedure was more generally satisfactory.

SUMMARY

Concussion was produced by applying adequate force to the cat's brain by means of a pendulum striking the head or a hydraulic system connected with the interior of the cranium. Respiration ceased; the canthus, pupillary-light, startle and pinna reflexes disappeared; the pupils dilated and the nictitating membrane retracted. Electrical stimulation within the brain by means of fine electrodes placed with the Horsley-Clarke instrument revealed that excitability of the intra- and extra-cranial portions of the cranial motor nerves and of the descending fibers from the cerebral cortex in the basis pedunculi were unimpaired

in concussion. Excitability of nerve-cell groups comprising cranial motor nuclei was impaired less frequently, to a smaller degree and for shorter intervals than excitability of supranuclear motor parts, such as brain-stem tegmentum, hypothalamus and motor cortex. The inspiratory center of the medulla oblongata behaved much like a cranial motor nucleus. Cessation of respiration and abolition of reflexes appeared not to be motor defects. Concussional alterations in function set in at the moment concussion was produced and functional recovery occurred gradually. A brief, often momentary, excitatory effect on motor neurons, apparently correlated with decreased thresholds, was sometimes observed upon concussion.

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THE EFFECTS OF RENIN AND ANGIOTONIN ON CARDIAC OUTPUT AND TOTAL PERIPHERAL RESISTANCE[1]

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Received for publication November 22, 1943

The effects of renin and angiotonin on cardiac output/minute, and consequently the share that cardiac factors play in their pressor action, remain questionable. Hill and Andrus (1) reported no effects on heart rate and an increase both in systolic discharge and minute cardiac output of heart-lung preparations. Results on cat hearts perfused with Locke's solution suggested that the coronary vessels are constricted. Lorber (2) confirmed these observations on completely isolated hearts perfused with blood and reported also a decrease in diastolic size and an improvement in oxygen utilization, work, and efficiency of the heart. Data as to how arterial and venous pressures changed were not given.

Since results obtained on isolated hearts are partly determined by the way the apparatus is arranged and since conditions are not quite comparable to those in the body, information as to changes which occur under natural conditions is desirable. Two such studies on man were almost simultaneously reported. Both groups evaluated systolic discharge and cardiac output from ballistocardiograms and made supplementary studies by roentgenographic methods. Bradley and Parker (3) found no appreciable decrease in diastolic size but a reduction in systolic discharge and considerable diminution of minute cardiac output, owing to a marked cardiac slowing. Wilkins and Duncan (4) obtained somewhat similar results but noted an increase in diastolic size and rise in venous pressure. Their designation of the condition as "myocardial failure" seems to stretch the term. Decreases in cardiac output in man have also been reported by Cournand, Rogers and Riley (5) and by Taylor and Page (6).

PROCEDURE. The question was reinvestigated by quantitative cardiometric methods on dogs under morphine-barbital anesthesia. The procedures used and the precautions required in such studies have been described in previous papers from this laboratory (7, 8). A few technical changes were introduced which simplified exact quantitative evaluations. For example, it was found possible and advantageous to apply the cardiometer without opening the pericardium, thereby at once simplifying its placement and eliminating a criticism of the method. By recording the form of curves just before, during and after use of renin or angiotonin, the freedom of curves from artifacts (8), particularly at the onset and end of systole, could be tested. Only curves which were either technically perfect or capable of easy correction were used. Right atrial pres-

[1] The expenses of this investigation were defrayed by grants from the Commonwealth Fund and the Rockefeller Foundation.

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sure was recorded simultaneously by a water manometer and a rise exceeding 1 cm. of water after application of the cardiometer served as a good criterion of a too snug fit of the membrane. Leaks would of course be made obvious in calibrations just before and after each observation. All calibrations were made with the beating heart in the cardiometer, as previously described (8).

Injections of renin and angiotonin, diluted to 5 cc. were made into a femoral vein, the doses depending on the preparation.[3]

The following notes serve to indicate the condition of our animals at the time of injection. The average mean arterial pressure was 110 mm. Hg, the minute

TABLE 1

Showing increases in various dynamic factors after renin and angiotonin

EXPT.	PREPARATION	DOSE	VENOUS PRESSURE	DIASTOLIC VOLUME	SYSTOLIC DISCHARGE	HEART RATE	CARDIAC OUTPUT	ARTERIAL PRESSURE	T.P.R. A.U. (INCREASE)	T.P.R. A.U. (CONTROL)
		cc.	*mm. H_2O*	*cc.*	*cc.*	*minutes*	*cc.*	*mm. Hg*		
1—A	Angiotonin (Lilly)	0.5	+4	+2	+0.3	0	+54 (3.3%)	+10	270	6,410
B	Angiotonin (Lilly)	1.0	+6	+4	+0.0	−12	−114 (6.9%)	+12	1,000	6,010
C	Angiotonin (Lilly)	2.0	+6	+5	+0.5	−6	+38 (2.4%)	+24	1,360	6,180
D	Renin (Lilly)	0.1	+5	+4	+0.5	−12	−30 (1.5%)	+14	720	5,380
E	Renin (Lilly)	0.2	+6	+3	0	−18	−153 (9.1%)	+15	1,360	5,700
2	Renin (Lilly)	0.3	+2	+7	−0.34	−8	−102 (?)	+56	7,250	5,273
3—A	Renin (Lilly)	0.5	+49	+4	−2.26	−20	−911 (49%)	+73	6,770	3,470
B	Renin (Lilly)	0.5	+14	+2	−0.81	−9	−190 (22%)	+43	8,300	10,750
4—A	Angiotonin (Lilly)	0.5	+2	+1	−0.6	+7	−41 (3.4%)	+22	1,730	5,900
B	Angiotonin (Lilly)	0.3	+6	+2	−0.2	0	+29 (2.4%)	+38	2,370	6,250
5—A	Renin (Lilly)	0.4	+5	+5	−0.3	−21	−134 (15%)	+36	7,180	11,820
B	Angiotonin S.M.A.	1 unit	+4	+6	−0.4	+9	−10 (1.2%)	+43	4,420	9,380

cardiac output averaged 2.47 L/min./sq.m., and the total peripheral resistance (T.P.R.) calculated from them ranged from 3700 to 7000 A.U.

RESULTS. Injections of renin and/or angiotonin were made in 14 dogs. In 6 of these renin or angiotonin injections were repeated at hourly intervals to test the effects of increasing and decreasing doses. In 8 of these angiotonin and renin were injected successively in order to compare their effects on the same animal.

An analysis of all records and the tabulated data revealed that, except for speed and duration of action, the two substances are similar in their dynamic actions. To conserve space, only enough data from a large tabulation are included in table 1 to illustrate the variety of effects and their dominant trends.

[3] We are indebted to Mr. J. L. Schmidt of the S. M. A. Corporation, Chagrin Falls, Ohio, and to Dr. I. Page of Ely Lilly Co., Indianapolis, Indiana, for the supplies of renin and angiotonin used.

As illustrated by experiments 1 and 3, progressively increasing doses of renin or angiotonin cause progressively greater pressor effects. Right atrial pressure increased only a trifle when smaller doses were used, but rose considerably with more potent doses (3, A, B). Regardless of venous pressure changes, the diastolic size *increased* in the majority of tests. Even with doses causing but small elevations of arterial pressure, some dilatation occurred. Contrary effects such as reported by Lorber were never observed. In many instances, a moderate decrease in heart rate probably contributed to the dilatation noted, but since it occurred also in instances in which no rate changes occurred (e.g., 1A, 4B), a direct effect on the myocardium must be inferred. The small changes in venous pressures could not have produced such effects.

With very small doses, the stroke volume showed only slight changes in the direction of a very slight increase or decrease. With more effective doses (Expts. 2, 3), generally those which elevated mean pressure more than 30 mm., a variable reduction occurred. Unquestionably, both agents depress the myocardium when given in sufficient quantities.

With small doses, cardiac rate generally decreases, as shown in the table. Apparently, the normal compensation, i.e., an increase in stroke volume as heart rate slows and diastolic volume increases, is lost or reduced in the depressed myocardium. The cardiac output/minute is consequently always decreased. With minimal doses of renin (e.g., expts. 1, 2, 4, 5B) the reduction was less than 10 per cent; with more effective doses (e.g., expt. 3) it was sometimes reduced to half. In the former the reduced output/minute was due entirely to slowing (expt. 1), in the latter reduction in stroke volume was dominant (expt. 3). These results are in agreement with those of most of the previous investigators. While the cause of the myocardial depression was not established, it is a fair inference that this is related to somewhat variable effects on coronary flow. The cardiometric changes certainly resemble effects of pitressin, a well established coronary constrictor.

On the basis of such cardiac changes, we may conclude that the rise in mean arterial pressure in animals gives a fair quantitative index of changes in total peripheral resistance following injections of renin and angiotonin, but only provided small doses—usually those causing elevations of 30 mm. or less—are used. Large doses cause erratic effects owing to variable reduction in minute cardiac output.

The question was further studied by calculating changes in total peripheral resistance in absolute units (A. U.) as in previous reports (8). In most of our animals the control resistance ranged between 5000–7000 A.U., which is somewhat higher than in less extensively operated dogs. As illustrated in experiments 1 and 3, the resistance increased with the dosage, as did the mean arterial pressure. When a previous state of increased peripheral resistance existed, equivalent doses of renin or angiotonin caused not merely a further increase but often an accentuation. This is illustrated in experiment 5A in which the effects of a previous test with a small dose of epinephrine had not fully passed off and in figure 3B, in which the vascular effects of a previous dose of renin

seemed to persist for fifty-eight minutes. For example, the results of the last experiment showed that a first 0.5 cc. dose of renin caused a pressor effect of 73 mm., while a second equal dose approximately an hour later, produced a pressure rise of 43 mm. This tendency to a pseudo-tachyphylactic response was obviously due to the fact that the depressant cardiac action of the first dose persisted and hence the gradual fall of blood pressure to approximately the control level, did not signify that the vascular effects induced by the first dose had disappeared. Calculations of T.P.R. showed that the second dose caused a greater increase in total peripheral resistance than did the first, but this was not mirrored in the rise of arterial pressure owing to further reduction in minute cardiac output.

The conclusion is that when significant cardiac depression is induced by renin or angiotonin, the pressor effects induced by successive doses do not offer reliable information with regard to the degree of peripheral constriction induced. This may be of importance in the study of tachyphylaxis and in comparing the relative potencies of different preparations by successive tests on the same animal. Since such cardiac effects are absent or minimal with doses which cause pressor effects which do not exceed 30 mm., it is suggested that as small doses as possible be employed in such studies.

SUMMARY

1. The effect of renin and angiotonin on cardiac output was studied by a refined quantitative cardiometer method.

2. Small doses cause insignificant changes in systolic discharge in the direction of slight increases or decreases, but the concomitant slowing reduces cardiac output/minute slightly. A study of changes in total peripheral resistance indicates that the pressor rise offers a reasonable indication of the magnitude of peripheral vasoconstriction.

3. More potent doses—generally those which cause a pressor effect of 30 mm. or more—result in a variable reduction in systolic discharge and together with cardiac slowing may reduce cardiac output/minute very significantly. In such instances, pressor effects underestimate the change in total peripheral resistance considerably.

4. Since such cardiac depression may persist after use of larger doses, the return of arterial pressure to control levels may not be a sign that its peripheral action has passed off. In all tests requiring repeated injections, it is recommended that doses be used which do not evoke pressor effects in excess of 30 mm. Hg.

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THE EFFECTS OF RENIN AND ANGIOTONIN DURING HEMORRHAGIC HYPOTENSION AND SHOCK[1]

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Received for publication November 22, 1943

Since the fundamental investigations of Goldblatt, Page, Houssay and their respective associates have elucidated in part the rôle of the kidneys in experimental hypertension and the mechanism by which renin becomes an effective agent, the possibilities have been suggested 1, that the renin–renin substrate–angiotonin–hypertensinase system represents a homeostatic mechanism for the control of normal arterial pressure, and 2, that an increased production of renin and subsequent development of tachyphylaxis occurs during hemorrhage. Thus, Hamilton and Collins (1, 2, 3), Sapirstein, Ogden and Southard (4, 5), and later Huidobro and Braun-Menendez (6), presented evidence that the renin content of dog's blood increases after hemorrhage. Hamilton and Collins (1, 2, 3), also found that with prolongation of a hypotensive state, animals became refractory to renin, or at least its pressor action was much diminished. They suggested that such tachyphylaxis could explain final failure of the circulation in shock. Sapirstein, Southard and Ogden (5) reported in addition that injection of blood containing renin substrate produced a pressor effect during post-hemorrhagic hypotension and this suggested that this substance may be depleted in hemorrhage and shock.

This paper deals with observations on the pressor responses and changes in peripheral resistance produced by renin and angiotonin at various stages of standardized hemorrhagic shock (7, 8), a method which permits an evaluation of the actions of renin and angiotonin in two states of post-hemorrhagic hypotension, viz., a state which *can be* and one which usually *cannot be* reversed by subsequent reinfusion of the animal's own blood. At first, an attempt was made to do this in "open chest experiments" in which cardiac output was determined cardiometrically as described in a previous paper (9). In this way, changes in total peripheral resistance, rather than pressor changes of uncertain origin, could be made a criterion of peripheral reactions. During many trials this did not prove entirely satisfactory owing to the difficulty of keeping such animals alive during the period of drastic hypotension. As Wiggers and Werle (11) had previously found, the use of artificial respiration and application of a cardiometer during anesthesia superimposes some lethal component which makes unsatisfactory the method for producing standardized hemorrhagic shock by graded bleeding. The animals either die prematurely of cardiac failure or they pass

[1] The expenses of this investigation were defrayed by grants from the Commonwealth Fund and the Rockefeller Foundation.

[2] Travelling Fellow of the Rockefeller Foundation.

into a state of shock not solely attributable to hemorrhage. Consequently, the most satisfactory experiments were those carried out on naturally breathing animals with minimal operative procedures. Fortunately, such experiments are fairly conclusive because, as was shown in a previous communication (11), pressor changes following injection of angiotonin and renin offer a fairly quantitative index of changes in peripheral resistance, provided doses are used which do not cause elevation of mean pressure in excess of 30 mm. Hg.

METHOD. Dogs anesthetized with morphine (ca 3 mgm./kilo) and sodium barbital (175 mgm./kilo) were used. The animals weighed from 9 to 13.5 kilos. They were operated upon with the minimum possible traumatism. The femoral mean arterial pressure was recorded by a mercury manometer, and central pressure pulses by the routine technique used in this laboratory. The animals were bled following the method described by Huizenga, Brofman and Wiggers (8) for the production of "standardized hemorrhagic hypotension and shock." Briefly, it consists in bleeding a dog from the femoral artery at a rate of approximately 50 cc./min. until mean arterial blood pressure is reduced to 50 mm. Hg and in maintaining this pressure level for 90 minutes. This has been found to be a reversible period. After this time the animal is bled again until a mean pressure of 30 mm. is realized and kept at this level for 45 minutes. This develops an irreversible state in approximately 75 per cent of animals, for when all the blood withdrawn into heparin is reinfused through a femoral vein at the end of these periods either a precipitate or somewhat delayed circulatory failure supervenes. These stages are schematically shown in figure 1. Renin and angiotonin were tested during the control period prior to hemorrhage (A), at the end of the 50 mm. hypotension period (B), at the end of the 30 mm. period (C), immediately after the reinfusion of blood (D) and at various intervals during the post-reinfusion period (E, F.). The quantities of renin and angiotonin solutions administered to different animals varied, as I used preparations of different origin,[3] but for reasons given in a previous paper the doses chosen were small enough to produce no more than a 30 mm. rise in mean arterial pressure during the control tests. The same dose was given in subsequent injections. The agents were diluted to 5 cc. and injected into a femoral vein. The blood withdrawn was received in a container with excess of 10 per cent heparin solution to eliminate any possibility of clotting and was kept in the ice-box at 7°C. until shortly before it was reinfused. This was done in order to minimize interactions of renin and renin substrate and the subsequent destruction of angiotonin (hypertensine) by hypertensinase.

RESULTS. The data presented first are based on observations on 10 naturally breathing dogs all of which, after reinfusion of blood, developed either a precipitate or delayed form of shock, as defined by Huizenga et al. (8). The schematic diagram of figure 1 illustrates experiments chosen to bring out the few inconsistencies which occurred rather than consistencies. Plots I, II and III repre-

[3] We desire to thank Dr. Irvine Page of Eli Lilly Laboratories, Indianapolis, and Mr. J. L. Schmidt of the S. M. A. Corporation, Chagrin Falls, O., for generous supplies of renin and angiotonin.

sent the rise in millimeter Hg of blood pressure resulting during different stages of the experiments from injection of angiotonin and renin at different stages. The white rectangles represent reactions to renin; the black ones to angiotonin. Circles indicate absence of reaction. Plot I illustrates reactions in precipitate and plot II in delayed shock to angiotonin and renin, both tested as described above. Plot III shows reactions obtained in two animals in which no renin

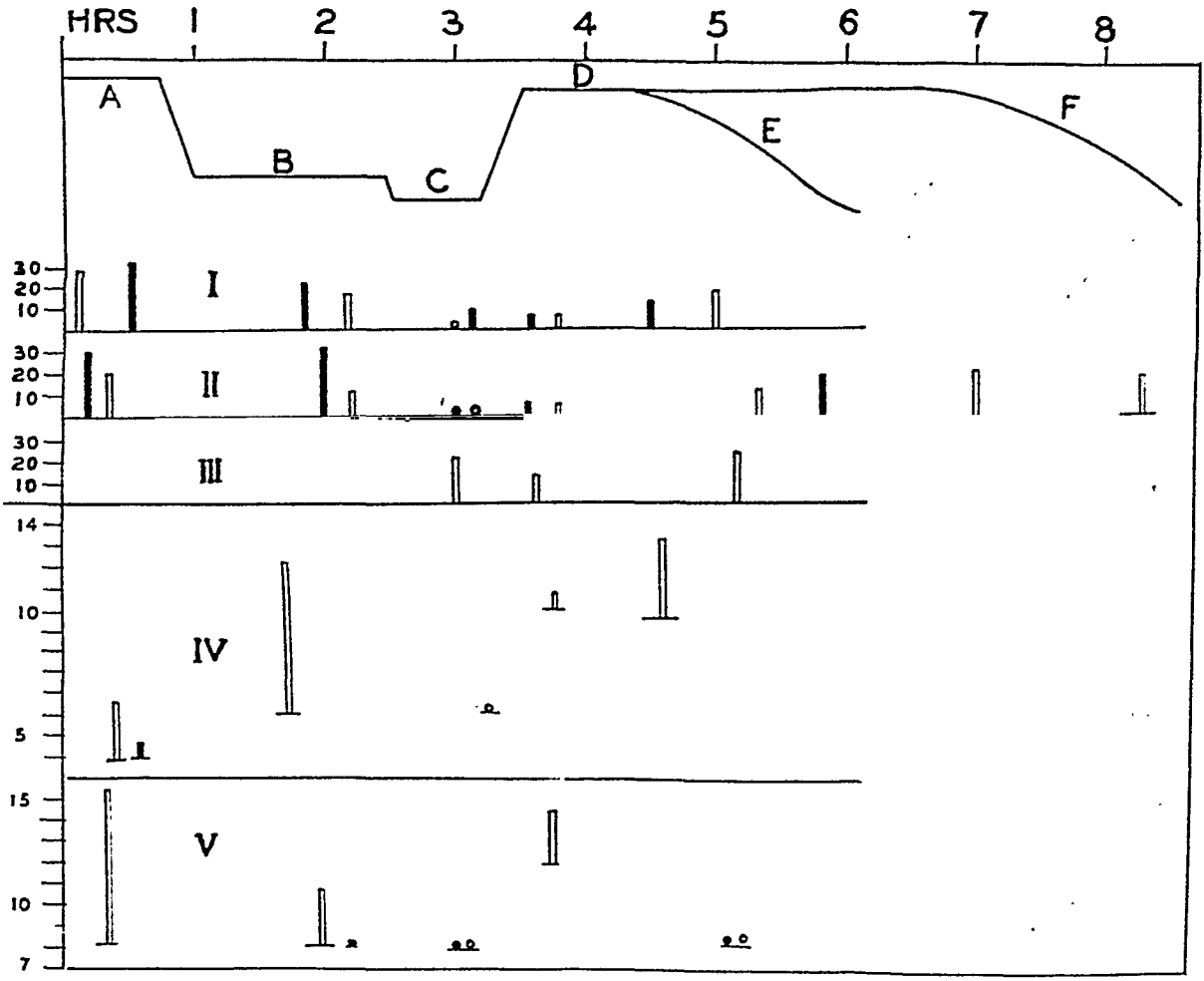

Fig. 1. Plots showing the response of renin (white rectangles) and angiotonin (black rectangles) during two stages of hemorrhagic hypotension, after reinfusion and shock. *Ordinates;* for I, II and III, rise of blood pressure in millimeter Hg; for IV–V, changes in total peripheral resistance expressed in A.U. $\times 10^3$. Discussion in text.

or angiotonin had been administered until the end of the 30 mm. period. This was done in order to check the possible development of an artificial tachyphylaxis in experiments of this type, despite the allowance of intervals which are supposed to obviate such actions.

In the eight experiments typified by plots I and II, the pressor responses to renin and angiotonin were generally reduced during the 50 mm. period, the exceptions being the instance shown in plot II. However, such reactions were

either completely abolished at the end of the 30 mm. period, or reduced to very questionable elevations of pressure.

Shortly after reinfusion of blood which presumably contained renin substrate (α globulin, Page; hypertensinogen, Braun-Menendez) and which essentially restored arterial pressures to control levels, some pressor response to both renin and angiotonin was generally found, but this increase was never great. However, the unexpected observations were made that in most of the experiments these responses became progressively greater, despite the fact that arterial pressures fell (plots I, II).

In two experiments illustrated by plot III in which no injections were made until the end of the 30 mm. period, renin caused a good response and after reinfusion this was much less. This raises some question with regard to possible retention of effects of previous injections. However, this would seem to be discounted by the fact that the pressor response improved later when the precipitate decline of pressure was well under way. Results from two of the most successful cardiometer experiments in which total resistance (TPR) was calculated are shown in plots IV and V. The bottom of the rectangles shows the TPR in absolute units (A.U.) at the time the injections were made and the height of the rectangles shows the magnitude of change. The results show again that the effects on TPR are abolished at the end of the 30 mm. period and, with the exception of the experiment shown in plot IV, are reduced during the 50 mm. period. This sequence occurred regardless of whether changes of TPR occurred at different times of the experiment (plot IV) or whether this happened to remain relatively constant, as in plot V. The tendency for reactions to increase in the post-infusion period was clearly seen in only two of the five experiments, one of which is shown in plot IV. However, it should be noted that recovery and sustenance of blood pressure were never as good as those "open chest" experiments, so that failure to observe this effect consistently is not necessarily significant. It is possible to state with confidence from experiments such as are shown in plot IV that the incrementing pressor reactions during the post-hemorrhagic period are a true peripheral effect and not due to an increase in cardiac output.

Discussion. Certain deductions can be drawn from the various reactions obtained:

1. No essential differences were found in the effectiveness of renin and angiotonin when injected in various periods of post-hemorrhagic hypotension, after reinfusion, and during subsequent circulatory failure. As nearly as can be estimated from experiments, renin *or angiotonin* in doses sufficient to cause definite pressor effects in control animals both become less potent during the 50 mm. period of hypotension and lose their potency toward the end of the 30 mm. period. When again tested 15 or 20 minutes after reinfusion the capacity to react both to renin and angiotonin is partially regained. These facts taken together offer no support for the conclusion that the lack of response to renin during the stage in which irreversibility develops is related to a possible deficiency of renin substrate.

2. When equivalent doses of renin or angiotonin were administered at progressively longer intervals after reinfusion of blood, the intensity of the pressor responses did not decrease again, but on the contrary generally tended to improve. In a few open chest experiments it could be shown that this was due to a greater augmentation of TPR. Since this occurred while circulatory failure, indicated both by declining arterial pressure and cardiac output/min., was in progress, it cannot be claimed that such failure is due to reduction in the renin substrate.

3. The finding that the intensity of the pressor response both to renin and angiotonin increased when tests are made at progressively longer intervals after reinfusion is not easy to explain. Several possibilities may be examined: *a*. The hypothesis that this is indicative of a progressive default of hypertensinase (angiotoninase) as shock develops, was considered. It is difficult to picture why such a hypothetical decrease in this enzyme does not begin to manifest itself during the 30 mm. period when the damage is being done. The fact that pressor responses both to renin and angiotonin are often completely lacking would be difficult to explain on such a concept. The chief objection to such a hypothesis was pointed out to me by Doctor Goldblatt: Hypertensinase (angiotoninase) is not a rapidly acting enzyme, like cholinesterase, for example; on the contrary, it acts so slowly that its presence or absence would have no discoverable effect on angiotonin administered intravenously.

b. The possibility that the decreasing and increasing pressor responses to renin and angiotonin may be due to changes in arterioles rather than in humoral mechanism cannot be overlooked. It is conceivable, for example, that vessels already constricted during post-hemorrhagic hypotension can contract no further, but as they become dilated during the development of circulatory failure they may contract again. Our "open chest" experiments showed no correlation, however, between the existing TPR and the magnitude of response. Thus, as shown in plot IV, the pressor response to renin after reinfusion increased, although TPR was practically constant; and in plot V it was later abolished when TPR had been reduced.

c. The possibility that the variable reactions are due to depression and recovery of central nervous mechanisms in our experiments must be considered in view of the recent findings of Page (11) that similar states of refractoriness to angiotonin develop after severe central nervous trauma and severe hypotension. If this is true, deterioration of central nervous function does not go hand in hand with circulatory failure of shock as is generally supposed. It is wise, however, not to invoke any such central mechanism until our ideas shall have become clearer with regard to their modus operandi.

It is necessary to conclude that these experiments have yielded no valid evidence in support of the suggestion that the mechanisms by which renin is activated is implicated in the development of circulatory failure which follows prolonged post-hemorrhagic hypotension and that the gradual recovery of response to renin and angiotonin during progressive circulatory failure remains unexplained.

SUMMARY

1. The effects of renin and angiotonin in doses causing a pressor response of 30 mm. Hg, or less, were determined during two stages of post-hemorrhagic hypotension and at various periods after reinfusion of the blood, i.e., during development of precipitate or delayed circulatory failure regarded as characteristic of shock.

2. In confirmation of other investigators, it was found that the pressor responses to *both* of these agents diminished and then disappeared during the prolonged hypotension, but recovered and increased progressively after reinfusion of blood despite the development of circulatory failure.

3. The results failed to support the suggestion that the mechanisms by which renin is activated is implicated in the development of circulatory failure and that the gradual return of response during progressive circulatory failure after reinfusion remains unexplained.

I desire to express my appreciation to Dr. C. J. Wiggers for guidance in this work and to Dr. Harry Goldblatt for advice in the interpretation of some of the results.

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ADRENAL CORTICAL COMPOUNDS AND *l*-ASCORBIC ACID ON SECRETING KIDNEY TUBULES IN TISSUE CULTURE[1]

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Received for publication December 4, 1943

The importance of adrenal cortical material on renal function has been indicated by Harrison and Darrow (1) who found that in adrenalectomized dogs there is a failure of the renal tubules to maintain concentration differences of certain ions between the urine and the blood plasma. This disappeared on the administration of adrenal cortical extract.

The present study was undertaken to ascertain the effect of several preparations derived from the adrenal cortex on the secretory activity of the proximal tubules of the chick kidney in tissue culture. The preparations, obtained through the courtesy of Dr. E. C. Kendall (Mayo Foundation), were Kendall's whole cortical extract, Kendall's crystalline Compound A (11-dehydrocorticosterone), Kendall's crystalline Compound E (17-hydroxy-11-dehydrocorticosterone) and Kendall's amorphous fraction.

The cultures consisted of teased fragments of the mesonephros from 9 day chick embryos prepared in the usual way in chick plasma and mounted on coverslips. Attention was centered on the proximal convoluted tubules. The conversion of the fragmented tubules in the cultures into closed tubular segments which become progressively distended with secreted fluid has already been described (2). Briefly, the closure occurs by a proliferation at the cut ends of the tubules with a resulting plug of epithelial cells which becomes converted, within 10 to 12 hours, into a single layer indistinguishable from the rest of the wall of the tubule. The distention of these tubular segments becomes increasingly apparent after 24 to 48 hours of incubation at body temperature. This accumulation of fluid and of phenol red has been used to test the action of various inhibiting and stimulating agents on kidney secretion (3, 4).

Compounds A and E, obtained in crystal form, were put into aqueous solution as follows: Compound A was dissolved in a small quantity of absolute alcohol and Compound E, in a mixture of propylene glycol and alcohol. These solutions were then diluted with Tyrode solution to obtain the concentrations used in the experiments. According to Kendall (5) the maximum solubility of Compound A in distilled water is 0.3 mgm. per ml., that of Compound E is 0.1 mgm. per ml. The whole cortical extract, which represented 75 grams of adrenal cortical tissue per ml. and the amorphous fraction which represented 150 grams per ml., were received in aqueous solution.

The controls contained the various diluents in amounts equal to those in the experiments and were consistently excellent. The Tyrode solution contained

[1] Aided by a grant from the International Cancer Research Foundation, Philadelphia, Pennsylvania.

sodium, potassium, calcium and magnesium chloride, and glucose; and was buffered with phosphate and carbonate to pH 7.8. In some experiments (those with the whole extract and with Compound E) the glucose was omitted with no change in the results observed. However, a certain amount of glucose was always present since the other ingredients of the culture medium consisted of whole plasma and embryonic extract.

Attempts to render the solutions sterile were unsuccessful since it was found that heating or passing the solutions through bacteriological filters removed their potency. However, the usual aseptic precautions in the preparation of the cultures were found to be sufficient to prevent appreciable bacterial contamination within the period of the experimentation.

The cultures were exposed to prepared solutions of the extracts in three ways: *a*, immersion of 48 hour cultures of the tubules for several hours in Tyrode solution containing the extracts; *b*, addition of a drop of plasma containing the extract to 24 or 48 hour cultures and allowing the plasma to clot; and *c*, mixing solutions of the extract with the plasma medium in which fragments of freshly teased proximal tubules were implanted for culturing. In method *a*, the coverslips, carrying the 48 cultures, were washed for one hour in several changes of Tyrode solution before transferring the cultures to the solutions containing the cortical extracts. This was done to eliminate possible traces of adrenal cortical material which might be present in the tissue and in the original plasma medium. This was later found to be unnecessary and, in methods *b* and *c*, the preliminary washing with Tyrode was omitted.

I. *Whole Cortical Extract.* a. *Immersion of Tyrode washed cultures (3 experiments, each with 10 cultures).* Cultures of 48 hours' duration containing partially distended tubules were immersed in aqueous solutions of whole cortical extract in concentrations of 1:1000 and 1:10,000 in Tyrode. Control cultures were immersed in Tyrode alone. All the solutions contained a trace of phenol red to aid in demonstrating the secretory activity of the tubules and also in checking the pH of the medium. The dishes containing the cultures were kept in an incubator at body temperature. At the end of 3 hours the cultures were removed from the solutions, remounted over depression slides and incubated for twenty hours further. Upon microscopic examination it was found that all the cultures receiving the cortical extract showed a much greater distention and a higher concentration of phenol red in the lumina of the tubules than did those of the controls. The two concentrations of 1:1000 and 1:10,000 under the observed conditions were found to be equally effective.

b. *Addition to 48 hour cultures (2 experiments, each with 15 cultures).* A drop of plasma containing the whole extract was superimposed on the original clot of a 48 hour culture, the concentration of the extract being adjusted so that it would vary from 1:2000 to 1:10,000 in different cultures. The experimental and control cultures were then incubated for twenty-four hours longer. Upon examination it was found that the cultures containing the cortical extract showed an increase in activity of the tubular segments similar to that observed in method a, with no observable difference in the different concentrations used.

c. *Addition to fresh preparations (3 experiments, each with 15 cultures).* Freshly teased segments of kidney tubules were imbedded in a culture medium containing the whole cortical extract in concentrations of 1:1000, 1:10,000 and 1:100,000, both with and without phenol red. The results agreed with those of the previous experiments. The cultures in 1:1000 and 1:10,000 concentrations showed marked activity, while those in 1:100,000 showed less but were still more active than the controls. The presence of phenol red has been previously found (6) to increase the secretory activity of the tubules, their distention being greater than when phenol red is absent. In the present experiments the presence of the cortical extract was found to increase the secretory activity of the tubules irrespective of whether the phenol red was present or absent.

II. *l-Ascorbic Acid Alone and in the Presence of Whole Cortical Extract.* A few experiments were made to test the effect of *l*-ascorbic acid on the tubular secretion and on the action of the cortical extract. The procedure was that of method a, in which 48 hour cultures were thoroughly washed in Tyrode prior to experimentation. This, as previously found (7), removed effective traces of *l*-ascorbic acid present in the normal plasma clot.

The washed cultures were divided into four lots and placed in the following solutions all of which contained phenol red in equal amount. The first solution contained whole cortical extract in a concentration of 1:10,000; the second, *l*-ascorbic acid, 1:10,000; the third, whole cortical extract 1:10,000 containing ascorbic acid in the same concentration; and the fourth, Tyrode alone, as control. The four groups were placed in the incubator at body temperature for three hours and then remounted over depression slides and incubated for 24 hours longer. The cultures from the two solutions containing the cortical extract showed an equally marked distention and accumulation of phenol red in the tubular segments. Those from the two solutions without cortical extract showed much less distention and less accumulation of phenol red indicating that the presence of the *l*-ascorbic acid had no stimulating effect on tubular secretion.

III. *Kendall's Compound E.* Compound E was introduced into the cultures according to the same three procedures used with the whole cortical extract.

a. *Immersion of 48 hour cultures (1 experiment with 6 cultures).* The cultures were immersed for 3 hours in Tyrode containing Compound E in concentrations of 1:100,000 and 1:500,000. After twenty hours' incubation the tubular segments were greatly distended with phenol red concentrated in them. They had developed into swollen cysts while the tubules in the controls showed normal distention only.

b. *Addition to 24 and 48 hour cultures (2 experiments each with 15 cultures).* Comparisons were made between the effect of 1:500,000 Compound E and of 1:10,000 whole cortical extract in plasma. After 20 hours' incubation, the tubules of cultures containing Compound E were found to be much more distended and with greater concentration of phenol red than those containing the whole cortical extract. Both showed more secretory activity than the controls.

c. *Addition to fresh prepartions (3 experiments, each with 18 cultures).* Freshly teased segments of the proximal tubules were placed immediately into culture media containing phenol red and solutions of Compound E in concentrations of

1:600,000 and 1:1,000,000. After 30 hours of incubation the experimental cultures showed much more distention and greater concentration of phenol red than the controls. No difference was noted between the effect of the two concentrations.

IV. *Kendall's Compound A (5 experiments, each with 12 cultures).* This compound was tested by all three methods in concentrations of what was calculated to be from 1:10,000 to 1:1,000,000. Concentrations up to 1:30,000 were definitely toxic. Slight toxicity persisted at 1:50,000 but below this concentration no effect of any kind was noted.

V. *Kendall's Amorphous Fraction (2 experiments, each with 17 cultures).* This compound was tested by methods b and c using concentrations from 1:1000 to 1:50,000. In concentrations up to 1:10,000 a slightly toxic effect was observed. The weaker concentrations exerted no observable effect except a tendency to render the luminal fluid slightly acid—pH 7.4 instead of the usual 7.8 of the normal chick mesonephros.

Discussion. It is of interest to note that of Kendall's two crystalline compounds, A and E, the secretory activity of the kidney tubules was stimulated by Compound E while no observable effect could be obtained with Compound A.

The significance of these results is indicated by comparing them with data obtained in this laboratory on the effect of several adreno-cortical steroids in perfusion experiments of the isolated hind-limbs of the frog (8). The perfusion fluid was a salt solution containing gelatin of a concentration sufficiently hypotonic to permit a progressively increasing edema of the legs. The addition of whole cortical extract to the perfusing fluid induced a marked reduction in the edema formation. Similar results were obtained with Kendall's Compound A. On the other hand, Compound E was found to be relatively ineffective until its concentration had been increased tenfold over that of the minimum effective concentration of A. Thus, the relative effectiveness of the two crystalline steroids in preventing fluid loss from the blood circulation is of a different order from that of the same compounds in enhancing renal secretion.

It is of considerable interest that the relative effectiveness of the two compounds on two different types of life processes, viz., fluid transfer across vascular walls and glandular secretory activity, can be referred to their different types of effectiveness on metabolic processes. Compound A, which is the more efficient agent in diminishing loss of fluid from the blood capillaries, is known to affect the metabolism of inorganic salts on which capillary permeability seems to depend (9). On the other hand, Compound E, which is the efficient agent in the renal secretory mechanism which is intracellular, is the agent known to enhance carbohydrate metabolism.

Another significant feature is that *l*-ascorbic acid which activates cell proliferation and growth (cf. 7) exerts no effect on renal glandular secretion.

SUMMARY

The secretory activity of the renal proximal tubules of the chick in tissue culture is stimulated by the presence of Kendall's whole cortical extract and of Kendall's crystalline Compound E (17-hydroxy-11-dehydrocorticosterone), but

not appreciably by Kendall's crystalline Compound A (11-dehydrocorticosterone), or Kendall's amorphous fraction. Likewise, *l*-ascorbic acid was found to be ineffective.

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SOME EFFECTS OF INCREASED FOOD CONSUMPTION ON THE COMPOSITION OF CARCASS AND LIVER OF HYPOPHYSECTOMIZED RATS [1]

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Received for publication December 15, 1943

It is well known that adult hypophysectomized rats, eating ad libitum, lose a large proportion of their body weight during the first few postoperative weeks. The weight loss is associated with, and possibly to a large extent due to, a marked voluntary decrease in food consumption. Lee and Ayres (1) have reported that such rats not only lose more weight than do intact animals fed the same amount of food (pair-fed) but also the operated animals lose a greater proportion of their nitrogen and water but less of their fat stores.

Previous experiments in this laboratory (2) have demonstrated that by forcibly increasing the food intake of hypophysectomized adult rats the body weight may be caused to increase above the preoperative level. The rate of gain is identical with that of pair-fed unoperated animals. Furthermore, providing an adequate food intake by tube feeding prevents the loss of serum albumin which uniformly occurs in hypophysectomized rats allowed to eat ad libitum. These findings suggested that interesting information might be obtained by studying the changes in carcass and liver composition in hypophysectomized rats caused to gain weight by forced feeding.

The results of such a study, reported in this paper, indicate that a large part, although not all, of the post-hypophysectomy changes in body and liver composition are referable to the decreased caloric intake and may be prevented by forcing the animal to metabolize larger quantities of food. However, such a procedure does not restore the animals to complete metabolic normality and it remains evident that factors other than caloric intake must also be considered.

EXPERIMENTAL. Male rats, 100 to 120 days old, were divided into four groups with body weights matching in the groups as nearly as possible. Two of the groups were hypophysectomized and the other two groups remained intact. Completeness of pituitary gland removal was checked at autopsy by examination of the sella under a high power dissecting microscope. In addition, testes, seminal vesicles and adrenal glands were weighed and the atrophy of these organs served as a check on the lack of hypophyseal secretion. The few cases in which the completeness of ablation was doubtful are excluded from the data reported.

In order to estimate the changes in composition resulting from the experimental procedures, one group of normal rats was sacrificed and the carcasses analyzed. The average values obtained were assumed to represent the average composition of the experimental animals at the start of the experiment.

[1] Aided by a grant, administered by Dr. P. E. Smith, from the Rockefeller Foundation.

One group of hypophysectomized animals was allowed to eat the stock diet ad libitum. For comparative purposes, the members of a group of intact rats of similar age and weight were subjected to a restricted food intake (stock diet) so that the loss of body weight simulated that lost over a like period by the operated animals eating the same diet ad libitum.

The members of another group of hypophysectomized rats were fed twice daily by stomach tube, the entire food intake being supplied in this manner. No food was given during the first 36 hours postoperatively because handling of the animals and passage of the tube immediately after operation appeared to delay healing of the operative wound. To determine the effect of the liquid diet per se, a group of intact rats was treated exactly as were the tube-fed hypophysectomized animals except that the operative procedures were omitted.

The method of tube feeding as well as the composition of the diets have been previously described (2, 3). The quantity of food given was the same as in earlier experiments, all tube-fed animals receiving the same amount of the food mixture. Each rat was provided with approximately 62 calories per day. Protein, carbohydrate and fat furnished 19, 35 and 46 per cent, respectively, of the total caloric intake.

All the experiments were conducted over a three week period. At the end of this time the animals were fasted for 18 hours and then autopsied while under amytal anesthesia. Livers were removed and treated as described below. The entire gastrointestinal tract was removed and discarded after being stripped of mesenteric fat which was returned to the carcass. Adrenals, testes, seminal vesicles, thyroids and kidneys were dissected out, weighed and returned to the carcass which was then ground in a meat chopper and weighed. Moisture was determined by drying the entire minced carcass to constant weight at 75°C.

The dried carcass was reground in the meat grinder and aliquots of the resulting uniform mixture were used for determination of total lipids and of total nitrogen.

Total lipids were determined by extraction with boiling ether. The ether extract was evaporated and the dry residue repeatedly extracted with petroleum ether. Insoluble material was removed by centrifugation or filtration, the solution transferred to a weighed vessel and after evaporation of the petroleum ether, the residue was dried to constant weight at 90°C. The percentage of total lipids was calculated from the weight of the dry residue.

Total protein content was obtained by multiplying total nitrogen (micro-Kjeldahl) of the dry carcass by 6.25. It is recognized that such values are somewhat high because no deduction was made for non-protein nitrogen.

Liver. After removal of an aliquot for lipid determination, the remainder of the liver was immediately frozen in CO_2 snow and rapidly weighed. The frozen liver was pulverized by use of the apparatus described by Graeser et al. (4), and aliquots of the resulting powder taken for glycogen and moisture determination. Glycogen was determined by the method of Good, Kramer and Somogyi (5). Moisture was determined by heating the pulverized specimen to constant weight at 90°C.

The dry residue obtained from the moisture determination was powdered in a mortar and aliquots of the powder used for determination of protein (6.25 × total N obtained by the microKjeldahl procedure).

Total lipids were determined on aliquots of the fresh liver by the method of Channon et al. (6). The chloroform soluble residue was repeatedly leached with petroleum ether, the solution filtered and after evaporation of the petroleum ether the residue was dried to constant weight.

RESULTS. *Carcass.* The pertinent data concerning ages, body weights and carcass compositions are summarized in table 1. The hypophysectomized animals eating ad libitum showed the usual weight loss amounting to 27.6 per cent of the initial weight during the three week postoperative interval. The intact rats subjected to restricted food intake lost an almost identical proportion (27.1 per cent) of the initial body weight.

The force-fed rats, on the other hand, gained weight, there being little difference in this respect between normal and hypophysectomized groups. It may be

TABLE 1

Weight and composition changes of rat carcass

	NO.	AT AUTOPSY		WEIGHT CHANGE AVERAGE		WATER	FAT	PROTEIN	UNDETERMINED
		Age	Weight						
		days	*gram*	*gram*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Normal—Ad libitum	28	103–139	242.9±4.6			62.40±0.43	11.59±0.58	21.00±0.22	5.00±0.15
Intact—Restricted food	11	125–130	202.5±4.1	−75.3	−27.1	66.20±0.55	4.19±0.82	23.31±0.43	6.30±0.15
Hypsect.—Ad libitum	14	125–139	187.4±6.9	−71.6	−27.6	62.49±0.70	10.34±0.96	21.74±0.29	5.43±0.12
Hypsect.—Force fed	12	112–139	263.3±5.9	+21.2	+8.8	53.34±0.90	23.98±1.16	18.04±0.26	4.65±0.11
Intact—Force fed	15	120–146	267.2±5.0	+20.6	+8.4	58.97±0.61	16.62±0.82	19.29±0.17	5.12±0.11

The ± values are for σ_M, calculated as $\sigma_M = \sqrt{\Sigma d^2/n(n-1)}$

mentioned at this point that the operated animals, fed adequate amounts of food, are distinguishable from intact animals only upon close examination. The roughness of the fur, the lack of body tone and the decreased spontaneous activity characteristic of hypophysectomized rats eating ad libitum are all largely prevented.

Comparison of the analytical values obtained from the different groups shows that considerable changes have occurred in the relative proportions of the constituents studied. However, a more direct measure of the changes during the experiment may be made by comparing the carcass compositions when expressed in terms of grams per 100 grams of initial body weight. Such a comparison is presented in table 2.

Our figures for untreated hypophysectomized rats and undernourished intact rats (table 2) show the same trend as do those of Lee and Ayres (1). The operated animals suffered a greater loss of water and of protein but a smaller loss of fat than did the intact animals whose weight has decreased to the same extent. Statistical analysis of the present data, however, indicates that the greater loss

of carcass fat by the underfed rats is significant[2] ($p = < 0.01$) but the differences in loss of water and of protein between these two groups are of doubtful significance ($p = 0.03$ and 0.04, respectively).

The changes in composition of the intact tube-fed rats are due entirely to the increase of the fat content from 11.59 to 18.17 grams per 100 grams initial body weight. The slight increase in water content is of doubtfiul signficance ($p = 0.07$), and the protein content is unchanged.

Feeding the same quantity of food to hypophysectomized rats also caused an increase in the carcass fat content. Whereas the fat stores of intact rats increased by 57 per cent those of the operated animals increased to 125 per cent over the initial value. The losses of body water and protein which occur in untreated hypophysectomized rats are largely prevented by the forced feeding, the content of these constituents being significantly greater ($p = < 0.01$

TABLE 2

Composition of rat carcasses

Expressed as grams per 100 grams initial body weight

	BODY WEIGHT CHANGE	WATER		FAT		PROTEIN		UNDETERMINED	
	per cent	*gram*	*P**	*gram*	*P*	*gram*	*P*	*gram*	*P*
Normal—Ad libitum		62.40±0.43		11.59±0.58		21.00±0.22		5.00±0.15	
Intact—Restricted food	−27.1	48.33±0.85	<0.01	3.09±0.63	<0.01	17.06±0.58	<0.01	4.59±0.10	0.10
Hypsect.—Ad libitum	−27.6	45.37±0.91	<0.01	7.68±0.79	<0.01	15.76±0.23	<0.01	3.94±0.12	<0.01
Hypsect.—Force fed	+8.8	57.99±1.00	<0.01	26.12±1.36	<0.01	19.61±0.30	<0.01	5.05±0.11	0.85
Intact—Force fed	+8.4	64.09±0.97	0.07	18.17±1.03	<0.01	20.97±0.29	1.00	5.57±0.14	0.02

The ± values are for σ_M, calculated as $\sigma_M = \sqrt{\Sigma d^2/n(n-1)}$

* P expresses the probability that the difference between the observed value and the initial value (that of the "normal ad libitum" rats) is due to random sampling.

in each case) than in the untreated operated animals but significantly less ($p = < 0.01$ in each case) than in the intact group eating ad libitum.

Liver. The decrease in liver weight after removal of the hypophysis is considerably greater than that of the body as a whole. The disproportionality is readily seen (table 3) when one compares the liver weight, expressed as per cent of body weight, of normal intact rats (3.49 per cent) to that of hypophysectomized rats eating ad libitum (2.82 per cent). An almost identical lack of proportion between the weight loss of liver and carcass is indicated for intact rats restricted to an inadequate caloric intake. One may calculate from the composition change that the slightly greater loss of weight by the livers of the operated

[2] The value P expresses the probability that the difference between two means is due to random sampling (17). Although a probability of 0.05 (5 chances in 100 that random sampling is responsible for the difference) is frequently accepted as a criterion of significance, a more rigorous criterion, i.e., $P = 0.01$, appears to be more desirable when small numbers of observations are compared.

animals is due to greater losses of water and protein. The total fat content of the livers of these two groups is almost identical.

The entire loss of liver weight following hypophysectomy is prevented by supplying an adequate food intake. The absolute liver weight as well as its ratio to body weight remains normal. Likewise, the proportion of water in the liver remains normal. The proportion of fat is significantly increased (initial value, 5.23 per cent; final value, 5.98 per cent, $p = < 0.01$). The total liver

TABLE 3

Weight and composition changes of rat liver

	NO.	FINAL BODY WEIGHT	BODY WEIGHT CHANGE	LIVER WEIGHT	LIVER WEIGHT / BODY WEIGHT	WATER	FAT	PROTEIN	GLYCOGEN
		gram	*per cent*	*gram*	*gram/100 grams*	*per cent*	*per cent*	*per cent*	*per cent*
Normal—Ad libitum.....	17	260.6		9.20±0.65	3.49±0.12	69.94±0.13	5.23±0.18	23.56±0.37	1.801* 0.349†
Intact—Restricted food..	11	202.5	−27.1	5.89±0.28	2.91±0.13	70.96±0.30	4.06±0.29	23.30±0.20	0.477
Hypsect.—Ad libitum....	11	191.6	−28.3	5.38±0.19	2.82±0.08	71.85±0.43	4.75±0.16	22.03±0.38	0.124
Hypsect.—Force fed.....	11	267.3	+6.5	9.40±0.47	3.52±0.16	69.98±0.23	5.98±0.18	22.22±0.24	0.726
Intact—Force fed.....	10	268.7	+9.8	9.07±0.35	3.36±0.08	69.72±0.14	5.55±0.10	22.61±0.11	1.260

The ± values are for σ_M, calculated as $\sigma_M = \sqrt{\Sigma d^2/n(n-1)}$

* Average of 24 rats of which 9 had not been fasted.

† Average of 15 rats fasted for 18 hours.

TABLE 4

Relation of organ weight to body weight

	NO.	BODY WEIGHT		LIVER WEIGHT			KIDNEY WEIGHT			TESTIS WEIGHT			ADRENAL WEIGHT		
					Weight/ body weight			Weight/ body weight			Weight/ body weight			Weight/ body weight	
		gram	*% chg.*	*gram*	*gram /100*	*% chg.*	*gram*	*gram /100*	*% chg.*	*gram*	*gram /100*	*% chg.*	*mgm.*	*mgm. /100*	*% chg.*
Normal—Ad libitum..	22	239.0		8.07	3.37		1.884	0.787		2.618	1.092		32.6	13.6	
Intact—Restricted food.....	11	202.5	−27.1	5.89	2.91	−14	1.775	0.877	+11	2.554	1.260	+15	31.7	15.6	+15
Hypsect.—Ad libitum.	11	191.6	−28.3	5.38	2.82	−17	1.134	0.595	−24	0.763	0.398	−64	10.7	5.6	−59
Hypsect.—Force fed...	11	267.3	+6.5	9.41	3.52	+5	1.351	0.507	−36	0.611	0.229	−79	12.8	4.8	−65
Intact—Force fed.....	10	268.7	+9.8	9.07	3.36	0	1.915	0.711	−9	2.845	1.059	−3	38.1	14.2	+4

fat content, although somewhat greater (by 17 per cent) than at the beginning of the experiment, is not increased by as great a proportion as that of the carcass fat (125 per cent higher than in the controls). The proportion of protein in the liver is significantly decreased (initial, 23.56; final, 22.22 per cent, $p = 0.01$) but it may be noted that the proportion of protein in the livers of the intact force-fed animals decreases to almost the same level. The total liver protein content, because of the slight increase in liver weight, is not significantly lower than at the beginning of the experiment and, in fact, is slightly greater than in the intact force-fed animals. It is therefore apparent that the changes in the liver which

follow hypophysectomy are almost completely prevented, as are those in carcass composition, by provision of an adequate supply of exogenous foodstuffs.

The glycogen values indicated in table 3 are in agreement with previously reported findings. The livers of hypophysectomized rats eating ad libitum contain less glycogen than do those of intact rats whose food intake is restricted. Similarly, force-fed operated animals have lower liver glycogen levels than do intact animals fed the same quantity of food.

The relationship of body weight to organ weight for several organs is summarized in table 4. Although the ratio of liver weight to body weight appears to be related to the nutritive state of the animal, the ratios for the kidneys, testes and adrenals, under the present experimental conditions, indicate little or no dependence on whether the animal gains or loses weight. The weights of these organs, as well as their relation to the body weight, directly reflect the presence or absence of hypophyseal function.

Discussion. Investigations of the metabolic activity of the anterior pituitary gland have frequently disregarded the rather severe self-imposed restriction of food intake which follows removal of this gland. The experiments of Lee and Ayres (1) demonstrated that hypophysectomized and intact rats, when given the reduced caloric intake determined by the appetite of the operated animals, differ in the proportion of water, protein and fat lost. It was further shown by Lee and Schaffer (7) that normal rats, treated with pituitary gland extracts containing growth hormone, gain more weight and deposit somewhat different proportions of body fat, protein and water than do pair-fed untreated rats. Although these and many subsequent findings (see (8) for references) have established the direct intervention of the anterior hypophysis in metabolic processes, the factor of appetite cannot be entirely discounted.

It has been shown (2) that forcing the hypophysectomized rat to metabolize amounts of food approximately isocaloric with that consumed by the intact rat eating ad libitum prevents the loss of body weight as well as that of serum albumin which ordinarily occurs after hypophysectomy (9). The present experiments demonstrate that the same procedure almost completely prevents the loss of carcass protein and water which occurs in the untreated hypophysectomized rat. The loss of fat is converted into an actual increase which entirely accounts for the body weight gain. The liver weight is maintained and the changes in liver composition are almost completely abolished.

It is therefore evident that if the caloric intake of the operated rats is maintained at approximately that of unoperated controls, the changes in composition and weight of the carcass and liver which follow hypophysectomy are not nearly so great as when appetite governs the amount of food eaten. This is of particular interest with respect to protein for great emphasis has been placed on the considerable loss of this component in the absence of growth hormone. It is widely believed that after hypophysectomy protein anabolism proceeds at a much slower rate than does its catabolism. However, the results of the present investigation indicate that if enough exogenous raw material is supplied, anabolic processes are sufficiently accelerated to greatly reduce the net loss of protein.

The results of Samuels, Reinecke and Ball (10) also demonstrate this point for in their experiments, conducted over a shorter period, the total quantity of protein was actually increased even though the body weight decreased slightly.

This can not be interpreted, however, as an indication that lack of growth in young hypophysectomized rats is only due to lack of appetite. That this is not the case is shown by the experiments of Samuels et al. (11) and also of this laboratory (12) in which young hypophysectomized rats, provided with an adequate food intake by force-feeding, although gaining in weight, nevertheless did not increase significantly in body length. In order to maintain growth after hypophysectomy it is necessary to maintain many functions including a positive nitrogen balance. In the present experiments, using adult rats, a positive nitrogen balance was not attained but the provision of an adequate food intake did greatly reduce the otherwise considerable nitrogen loss.

The excessive fat deposition by the force-fed hypophysectomized rat is also of interest. Samuels, Reinecke and Ball (10), feeding a diet high in carbohydrate and low in fat, observed a similar, though smaller, fat deposition. These authors interpret their finding as being due to a combination of a rapid conversion of carbohydrate to fat with a coincident disturbed mobilization or metabolism of the deposited fat. The fact that the total energy metabolism of these animals was considerably lower, without greater loss of weight, than that of other groups is ignored. It has been recognized, at least since 1912 (13, 14, 15, 16), that the metabolic rate of hypophysectomized animals is subnormal. This at least partially explains the greater fat deposition by hypophysectomized rats provided with a caloric intake adequate for normal animals as well as the smaller fat loss of hypophysectomized rats eating ad libitum than of pair-fed intact controls.

In the experiments of Samuels et al. (10), hypophysectomized rats fed a high fat and low carbohydrate diet did not deposit more carcass fat than did the pair-fed controls. Neither did these animals show the decreased metabolic rate usually found after removal of the pituitary gland. The relation between fat deposition and metabolic rate is, therefore, positive but the explanation of the *normal* metabolic rate of the operated animals on the high fat diet is lacking.

It is also pertinent, in this connection, to point out that our data for liver fat do not agree with those of Samuels et al. (10). The liver fat values reported by these authors do not correlate with the carcass fat content, whereas in our experiments the liver fat level in every case definitely reflects the carcass fat content.

Although the present experiments establish the fact that decreased food consumption plays an important rôle in the weight and composition changes noted after hypophysectomy, they do not in any way disprove the participation of the hypophysis in intermediary metabolic processes. The very fact that the hypophysectomized animals suffers from loss of appetite shows that profound metabolic changes have occurred. As is well recognized, the post-hypophysectomy syndrome is polyglandular in character and the individual components are difficult to isolate. Loss of appetite may be a function of deficiency of another of the glands effecting metabolic processes. Adequate evidence (8) is available, however, to establish the direct involvement of the anterior hypophysis in such processes.

SUMMARY

Hypophysectomized adult rats, forcibly fed quantities of food approximately isocaloric with those consumed by normal rats, gain instead of losing weight. Carcass analyses show that the large losses of protein and water occurring after pituitary gland removal are nearly completely prevented by giving the increased food intake. The actual gain in weight is due entirely to excessive fat deposition. Post-hypophysectomy changes in weight and composition of the liver are also nearly completely prevented but the weight losses of the kidneys, testes and adrenal glands are not affected by the increased food intake.

The author wishes to acknowledge the technical assistance of Miss Dorothy Wangerin. Thanks are also due to Miss Betsy Conant and to Dr. J. H. Leathem for hypophysectomizing the rats used in the experiments.

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THE EFFECT OF ADRENALECTOMY ON HEAT PRODUCTION IN YOUNG PIGEONS

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Received for publication December 13, 1943

It is known that heat production is maintained at a normal level in the absence of medullary adrenal tissue, but the effects of loss of the cortical tissue on heat production are not entirely clear. Most reports indicate that in mammals the loss of cortical hormones leads to an early decrease of the metabolic rate. The older literature has been reviewed by Grollman (1). Brownell and Hartman (2) noted, however, that sodium salts, like other treatments (cortin, sodium factor and desoxycorticosterone acetate) which maintained adrenalectomized dogs in good condition, supported a normal metabolic rate. In adrenalectomized dogs maintained on cortical fractions these authors also demonstrated that the specific dynamic effect of food is markedly delayed.

Riddle and Smith (3) made a preliminary report on the effect of adrenalectomy on the respiratory metabolism of adult pigeons. Repeated tests on 12 operated adults seemed to show only a slight effect (−6 per cent) on heat production, and no clear effect on the respiratory quotient. But careful autopsies made on the longest-lived survivors of that group of birds, and some additional experience, cast doubt on the completeness of the operation in some of the birds used in that series of tests. Thereafter our studies were limited to immature pigeons of a large breed. In these birds this operation can be performed readily and completely, and after adrenalectomy the birds can be maintained on a salt mixture long enough to permit measurement of their rate of heat production.

METHODS AND MATERIALS. The adrenals were removed under nembutal anesthesia in two operations separated by an interval of one week. Each adrenal was removed in one piece by blunt dissection, and the completeness of every operation was checked by histological examination of any questionable tissue. Prior to the second operation each bird was injected with 1 mgm. of desoxycorticosterone acetate in corn oil. After this operation each bird was fed, twice daily, a gelatin capsule containing 0.75 gram NaCl and 0.125 gram $NaHCO_3$. The birds used were all Carneau pigeons at 7 to 10 weeks after hatching. The average time of survival of nearly 150 birds thus operated was 9 days. Birds in terminal stages were not used, and all measurements of heat production were made at 2.5 to 7.5 days after the second adrenal was removed.

Though one and the same bird could not be subjected to all types of tests, many birds were measured in two or more of the following periods or states: before operation, fasted and not fasted; after removing one adrenal, fasted and not fasted; after removing the second adrenal, two grades or degrees of fasting. In order to standardize the nutritive state every bird was force-fed enough of a grain mixture to provide its crop with 15 grams food (and 15 cc. of water) at a

known number of hours preceding each test. Although 24 hours after food is certainly an adequate fasting period for intact pigeons it is probably inadequate for adrenalectomized pigeons. Water was withheld beginning 5 hours before measurements, and the second capsule of the salt-mixture was omitted on days when measurements were to follow at night. All tests were made at a temperature of 30°C., at night, in a closed-circuit multiple-chamber apparatus, under all conditions earlier described by Benedict and Riddle (4). These conditions included keeping the birds in a large constant temperature glass cage, at 30°C., from the time they were force-fed to the moment they were placed in the metabolism chamber.

The "critical temperature" for normal adult pigeons is 30°C. (4); it remains undetermined whether this is also true for a pigeon deprived of its adrenals. Moreover, other unpublished data (Riddle and Smith) indicate that normal

TABLE 1

Heat production (at 30°C.) in young Carneau pigeons before operation (control) and 2.5 to 6.5 days after removal of one adrenal only

	UNOPERATED				SAME BIRDS AFTER REMOVING ONE ADRENAL				
	Number of birds in group	Average calories per kilo per hour	Respiratory quotients		Number of birds in group	Average calories per kilo per hour	Per cent gain or loss	Respiratory quotients	
			Value	Number				Value	Number
Non-basal values 5–12 hrs. after feeding	7	4.97	0.81	2	7	4.84	−2.6	0.79	4
	4	4.62	0.74	2	4	4.99	+3.1	0.89	2
	2	4.19			2	4.55	+7.2	0.83	2
	Avg. 13	4.74	0.78	4	13	4.84	+2.2	0.83	8
Basal values (24 hrs. after feeding)	6	4.39	0.71	4	6	4.35	+0.96	0.69	2

young pigeons of the breed used here produce as little heat at 25°C. as at 30°C. In view of the known sensitivity of adrenalectomized animals to cold, however, it seemed advisable to make these measurements at the upper limit of the zone of thermal neutrality. Heat production was calculated from the oxygen consumption on the assumption that the respiratory quotients obtained were non-protein quotients.

Results. Non-basal values for heat production, at 5 to 12 hours after taking food, were obtained from 13 birds both before and after the removal of one adrenal (table 1). The average values from the two groups of tests differ by only 2.2 per cent. Values obtained on 6 birds at 24 hours after food differ by only 0.96 per cent. These data show that the removal of a single adrenal did not measurably affect the metabolism.

Table 2 lists tests made on birds after one adrenal was removed and again on the same (or other) birds at 3 to 8 days after removal of the other adrenal. Non-basal values, at 5 to 12 hours after food, were obtained at both stages on the same

37 birds; the second measurements here showed an average reduction of 10.4 per cent, and a reduction was observed in all of the seven groups included in this test. The respiratory quotients obtained averaged 0.84 from birds with one adrenal, and 0.81 from birds with no adrenal. Measurements which are known to give basal values (24 hrs. after food) for intact pigeons were obtained from 26 birds after one adrenal was removed, and again after removing the other adrenal; the latter measurements indicate an average *increase* in heat production of 6.9 per cent, and an increase was observed in all of the five groups of birds included in the test. Respiratory quotients from the two series averaged 0.70 and 0.73, respectively. Additional measurements were obtained either after removing both adrenals (15 tests, non-basal; 24 "basal"), or after removing only one adrenal (44 tests, non-basal; 18 basal), but not on identical birds at both of these

TABLE 2

Heat production at 30°C. in young Carneau pigeons after removal of one adrenal (control) and at 2.5 to 7.5 days after complete adrenalectomy

After complete adrenalectomy birds were given, twice daily, gelatin capsules containing 0.75 gram NaCl and 0.125 gram $NaHCO_3$.

	ONE ADRENAL REMOVED				COMPLETE ADRENALECTOMY				
	Number of birds in group	Average calories per kilo per hour	Respiratory quotients		Number of birds in group	Average calories per kilo per hour	Per cent gain or loss (+, −)	Respiratory quotients	
			Value	Number				Value	Number
Non-basal (?) values (5–12 hrs. after feeding)									
Same individuals.....	37	4.89	0.84	22	37	4.38	−10.4	0.81	26
Different individuals....	44	4.75	0.83	22	15	4.58	−3.6	0.78	9
Basal (?) values (24–26 hrs. after feeding)									
Same individuals.....	26	4.43	0.70	15	26	4.73	+6.9	0.73	18
Different individuals....	18	4.46	0.71	10	24	4.66	+4.5	0.73	16

stages. These data from unmatched individuals in the two series of tests tend to confirm the results obtained on identical birds before and after complete adrenalectomy.

Some data concerning the time of occurrence of the specific dynamic action (S.D.A.) of food were obtained from 6 pigeons (table 3). When these 6 birds retained one adrenal their average heat production at 7 hours after food was almost as high as that for the highest group listed in table 2. When both adrenals were removed from these birds their average heat production at 7 to 8 hours (two tests) after food was almost as low as the lowest of comparable groups in table 2; at 26 hours after food, however, their average heat production was 6.1 per cent higher than it was at 7 to 8 hours after food. Though these data provide no evidence for the complete absence of S.D.A. of food at 7 to 8 hours in adrenalectomized pigeons, they do indicate that at 26 hours this effect was 6.1 per cent greater than it was at 7 to 8 hours.

It seems well to provide additional data which suggest that the S.D.A. of food, though probably spread over a longer period, temporarily increases the rate of heat production of normal pigeons much less than it does in mammals. The data of table 4 were obtained over a period of five years on Carneau pigeons of the same age utilized in the present study. Uniformly these birds were fed 15 grams of mixed grain at 2 to 11 hours (or at 24 hrs. for basal values) before the measurements were started; but it should be understood that the measurements themselves extended over a further period of about 6 hours and that, as always, only the *lowest* values obtained (two to four) were utilized as true measures of rate of heat production. This use of minimum values which check each other is indispensable in the determination of basal metabolism, but it doubtless tends to mask the full extent of the S.D.A. Again, for tests of the point of maximum S.D.A. it is perhaps desirable to use periods of measurement somewhat shorter than 6 hours. For these two reasons the values shown in table 4 are probably too low but, as this was measured at all temperatures in groups of young Carneau pigeons, the S.D.A. of 15 grams of mixed grain was of the order of 9 to 14 per cent.

The rectal temperatures of 15 adrenalectomized birds were obtained at the conclusion of their metabolism tests. These data indicate that, following about 30 hours at a temperature of 30°C., there was little or no change of body temperature as a result of the loss of the adrenals; also, there was probably no significant difference between body temperatures of those operated birds which were measured at 5 to 12 hours and those measured at 24 hours after food.

Discussion. Heat production in pigeons measured at 30°C. has been shown to change very little when measured at 2.5 to 7.5 days following removal of the adrenals. The direction of the change, however, was found to differ in an unexpected way according to whether the last food was consumed at 5 to 12 hours, or at 24 to 26 hours, before measurement; the supposedly "basal" values—at 24 hours after food—are about 7 per cent higher, and the values at 5 to 12 hours after food are 4 to 10 per cent lower, than in birds still possessing one adrenal. This confusing result at first appears to support the paradox of a "basal" heat production (4.73 Cal./kilo/hr. for 26 tests) definitely higher than the non-basal value (4.38 Cal./kilo/hr. for 37 tests). We should now consider the probability that this anomalous result is a consequence of a delay of the specific dynamic effect of food in the absence of the adrenals.

A decreased rate of absorption of glucose from the intestine of adrenalectomized rats (5), and some ability of cortin to restore the rate of absorption (6), have been reported and confirmed. It also seems to have been established (7, 8) that the rate of absorption of longer chain fatty acids is decreased in adrenalectomized rats. Brownell and Hartman (2) observed in adrenalectomized dogs maintained on cortical fractions a marked decrease in the rate of development of the S.D.A. of ingested dextrose, protein and fat. In the normal dog the high point in the metabolism after 40 grams of dextrose was attained after 20 minutes, but this point was reached only after 5 or more hours in an extract-maintained adrenalectomized dog; for protein the time of maximum effect was delayed from 45 minutes

to 2 hours. The authors attributed this delay partly to a decreased rate of absorption and partly to a disturbance in the rate of conversion of dextrose to glycogen in the absence of the adrenals. This same interpretation seems applicable to the results obtained in the present study; and indeed it would seem to be applicable in an emphatic form, since our birds were maintained on salts instead of cortical hormone. The complete digestion and absorption of grain in normal pigeons probably requires a period of 10 to 19 hours; and, in some pigeons, as much as 20 to 24 hours is required to obtain a respiratory quotient of approximately 0.72 (4). Delayed digestion, absorption and glycogenesis in adrenalectomized pigeons might therefore lead to a specific dynamic effect at 24 hours after feeding and also reduce or prevent this effect at 5 to 12 hours after feeding. This situation was only partly appreciated, however, while the tests were being conducted. Both the apparent increase (6.9 per cent) in the equivocal "basal" heat production, and the decrease (10.4 per cent) in the dubious "non-basal" heat production, resulting from removal of the second adrenal, would seem to have this

TABLE 3

Data from 6 pigeons for heat production (at 30°C.) after removing one and both adrenals, and at various periods after food when completely adrenalectomized

CONDITION OF BIRDS	HOURS AFTER FOOD	CALORIES PER KILO PER HOUR	RESPIRATORY QUOTIENTS	
			Value	Number
One adrenal removed	7	5.08	0.93	1
Both adrenals removed	7	4.11	0.88	4
Both adrenals removed	8	4.26	0.80	4
Both adrenals removed	26	4.44	0.75	4

same simple explanation. In other words, the length of fast necessary for *basal* measurements in normal pigeons is not applicable to adrenalectomized pigeons. The several heat production values obtained, in addition to the direct tests of this point on 6 birds listed in table 3, tend to support this interpretation. Values obtained from completely adrenalectomized birds fasted 5 to 12 hours (4.38 and 4.58 Cal.) are clearly equivalent to average values from birds possessing one or both adrenals but fasted 24 hours (4.25, 4.35, 4.39, 4.43 and 4.46 Cal.); values from completely adrenalectomized birds fasted 24 hours (4.73, 4.66 Cal.) are close equivalents of average values from groups of birds having one or both adrenals but measured at 5 to 12 hours after feeding (4.74, 4.84, 4.89 and 4.75 Cal.).

The average daily food consumption of 12 birds was measured for 10 days following complete adrenalectomy and found to be only 7 grams. Unoperated controls consumed 31 to 35 grams daily. This marked reduction of food intake during a period of five days—the average time between complete operation and measurement—would be expected to produce a reduction of the metabolic rate. The daily water intake (120 cc.), however, due to salt effect, was double the usual quantity and this doubtless involved an increased expenditure of energy. The

body weight of several of these birds was found to show an average decrease of about 1 per cent per day following removal of the second adrenal (9). This suggests that all of the adrenalectomized birds may have been burning some of their own body protein at the time their heat production was measured. The special feeding of 15 grams of grain at 24 or at 5 to 12 hours before the metabolism tests may or may not have tended to spare this body protein. But if body protein was being burned, and particularly if it was being burned by the operated birds to an unequal extent at the two periods of fasting, that fact is of considerable significance. In that case any interpretation of the respiratory quotients becomes doubtful, and our use of them as non-protein quotients in calculating the caloric value of oxygen might involve an error of 5 to 10 per cent. Some of the values shown for adrenalectomized birds would then be too large by that amount.

TABLE 4

Heat production in normal young Carneau pigeons in the post-absorptive state (24 hrs. after food) compared with that of the same or similar birds at 2–11 hours after consuming 15 grams of mixed grain

Measurements made at various temperatures, and respiratory quotients obtained in one-half of all tests.

TEMPERATURE	24 HOURS AFTER FOOD			2–11 HOURS AFTER FOOD			
	Number of birds	Average		Number of birds	Average		Per cent increase (S.D.A.)
		Calories per kilo per hour	R.Q.		Calories per kilo per hour	R.Q.	
°C.							
30	128	4.25	0.72	162	4.79	0.82	+13.1
25	21	4.29	0.71	20	4.68	0.81	+9.1
20	28	4.56	0.73	26	5.19	0.93	+13.7
15	15	4.75	0.73	4	5.31	0.88	+11.8

The respiratory quotients obtained from adrenalectomized birds at the two periods after feeding seem opposed to the interpretation discussed above. It has been noted, however, that these quotients can not provide positive evidence on the point in question because of basic uncertainties involved in such quotients, and because these values were obtained from birds without adrenals and maintained without cortical hormone. Fisher (10) showed that 42 to 50 per cent of nitrogen ingested by normal homer pigeons is excreted as uric acid; also that approximately 24 hours elapse between the ingestion of food nitrogen and the excretion of the corresponding uric acid. Henry, Magee and Reid (11) noted that in fowls quotients lower than 0.70 resulted from the oxidation of protein. Their calculations further showed that the oxidation of the amino acid, alanine, to urea gives a quotient of 0.833, but when the end product is uric acid the quotient is 0.667.

The foregoing data show that in adrenalectomized pigeons truly basal heat production can not be obtained at the same period of fasting which it is necessary to use for intact animals. Probably this applies also to mammals. The present

instance moreover suggests that the removal of such organs as adrenals, thyroids and hypophysis may markedly change both the zone of thermal neutrality of the animal and the amount of time which must elapse between the taking of food and the attainment of the post-absorptive state.

The results of this study are in agreement with those of Brownell and Hartman who reported that any treatment—including sodium salts—which maintains an adrenalectomized dog in good condition also supports a normal metabolic rate.

CONCLUSIONS

The heat production at 30°C. of 102 salt-maintained young pigeons at 2.5 to 7.5 days after complete adrenalectomy, and not in terminal stages, showed little or no change directly due to loss of adrenal hormones. Since adrenalectomy was found to alter the conditions under which basal measurements must be made, truly basal values were probably not obtained. The metabolism of the operated birds was probably nearly basal at 5 to 12 hours after food, but apparently a specific dynamic effect was obtained at 24 hours after the ingestion of food.

Small indirect effects on heat production, apparently associated with delayed absorption and specific dynamic effect of food, were observed. Only when these indirect effects are overlooked do the results seem to indicate a paradoxical decrease of 4 to 10 per cent in oxygen consumption at 5 to 12 hours, and an increase of 5 to 7 per cent at 24 hours, after the ingestion of 15 grams of food.

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THE EXCRETION OF SULFANILAMIDE AND ACETYLSULFANILAMIDE BY THE HUMAN KIDNEY[1,2]

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Received for publication December 9, 1943

Sulfanilamide and acetylsulfanilamide are excreted by the kidneys of some animals by different mechanisms. In both the rabbit and the dog sulfanilamide is apparently filtered through the glomeruli and partially reabsorbed from the glomerular filtrate as it passes down the tubules (1, 2). On the other hand, available evidence indicates that acetylsulfanilamide is excreted by the rabbit kidney solely by glomerular filtration (2). This made it appear desirable to study the excretion of these compounds by the human kidney.

In the present study the clearance method was used to determine the mode of excretion of sulfanilamide and acetylsulfanilamide in human subjects. Renal clearance determinations of these two compounds were made simultaneously with inulin in an unselected group of adults. The group included not only those having normal kidney function but also several who had definitely decreased kidney function so that a wide range of clearance values was obtained.

METHODS. Each subject was given 1500 cc. of water to drink during the two hours preceding the test. This produced a urine flow of from 2 to 12 cc. per minute during the test. A catheter having two separate lumina throughout its entire length (one for inflow and one for outflow) was inserted in the urinary bladder. It remained there throughout the test. An irrigation apparatus was attached to the inflow channel of the catheter and by means of this, complete urine collections were obtained by irrigating the bladder with measured volumes of physiological saline at the end of each clearance period. The urine excreted plus the irrigation fluid was collected through the second channel of the catheter. Pressure was applied with the hand on the lower anterior abdominal wall to insure complete emptying of the bladder. Experiments showed that all of the irrigation solution could be recovered from the bladder.

Sulfanilamide was administered orally. The dose of this compound varied from three to five grams and was given three hours before the clearance periods were started. In a few cases acetylsulfanilamide was administered orally to the subjects in doses of from 3 to 6 grams and was given four hours preceding the test. The plasma concentrations of sulfanilamide and acetylsulfanilamide which were obtained during the tests varied from 1 to 15 mgm. per cent and from 1 to 5 mgm.

[1] This study was made in partial fulfilment of the requirements for the degree of Doctor of Philosophy by T. A. Loomis.

[2] This investigation was aided by a grant from the Junior Board of the Buffalo General Hospital.

per cent respectively. Inulin[3] was administered intravenously. A 10 per cent stock solution of inulin was added to pyrogen-free physiological saline so that the final concentration was 1 gram per 100 cc. In order to obtain the proper plasma concentration of inulin (which varied in the series from 10 to 60 mgm. per cent) the solution was allowed to run into the vein by the drip method at a rate of 10 to 15 cc. per minute for a period of fifteen minutes before the test was started. Following this the rate was maintained at about 3 cc. per minute during the experiment.

Following the period of rapid infusion the clearance tests were started. The bladder was washed with 200 cc. of physiological saline solution and the washings were discarded. In exactly fifteen minutes the bladder was washed again with exactly 200 cc. of the saline solution and the washings were added to the urine formed during the fifteen minute period. The urine bottle was then replaced with a second bottle, and at the end of the subsequent fifteen minute period the bladder washing procedure was repeated. Four similar clearance periods were obtained on each subject. At the midpoint of each period a sample of venous blood was taken. Although potassium oxalate was used in a few of the cases, heparin was the anticoagulant usually employed. The blood samples were centrifuged immediately and the plasma separated from the cells.

Both the plasma and urine samples were analyzed by the same methods following protein precipitation by the use of Somogyi's zinc sulfate and sodium hydroxide precipitating agents (3). The method used for the determination of sulfanilamide and acetylsulfanilamide was that developed by Bratton and Marshall (4). Inulin was determined by the method described by Hubbard and Loomis (5). Experiment showed that recoveries of the added compounds from plasma and urine following the use of the zinc precipitating agents were equal to those obtained when either trichloracetic acid or phosphotungstic acid was used. Furthermore the presence of these compounds in a common filtrate did not interfere with the determination of any one of them.

The maximum clearance formula, UV/P, in which U and P represent the urine and plasma concentrations respectively and V the urine flow in cubic centimeters per minute, was used for the calculation of the clearances. In most of the cases the urine flow was greater than 2 cc. per minute.

[3] Pfanstiehl's C. P. inulin was treated with Norit charcoal, recrystallized twice from alcohol, dissolved in pyrogen-free distilled water and filtered while hot through a Seitz E. K. filter. A 10 per cent solution of the compound was stored in two-ounce bottles which had rubber diaphragm stoppers. The solution was biologically tested for the presence of pyrogens by the following procedure: Samples of the solution were administered by vein to rabbits in one dose equal to ten times the amount which was received by the humans per kilogram of weight. Rectal temperatures were taken every fifteen minutes for one hour previous to the injection and for three hours following the injection. An increase in rectal temperature of more than 2 degrees C. within the three hour period was taken as evidence of the presence of a pyrogen in the solution. Solutions which showed pyrogen activity were discarded. In the human subjects rectal temperatures were taken before and after the clearances were determined. In no case did a subject show evidence that the infusion solutions contained a pyrogen.

RESULTS. A total of 82 simultaneous clearance periods of sulfanilamide, acetylsulfanilamide and inulin were obtained on 21 different subjects. The series included 17 females and 4 males. Simultaneous clearances of inulin and acetylsulfanilamide were obtained on an additional 4 subjects (2 males and 2 females) making a total of 96 simultaneous clearances of these two compounds. Statistical analysis of the data is presented in table 1. The ratios between the means of the various clearances are given in table 2. The clearances of the compounds which were studied were found to be relatively independent of the urine flow. This is shown in figure 1. Since the series of subjects includes some with decreased kidney function, the mean clearances which are presented in table 1 do not represent an average for normals. In those subjects who showed no clinical evidence of kidney deficiency, the mean clearances of inulin, sulfanilamide and

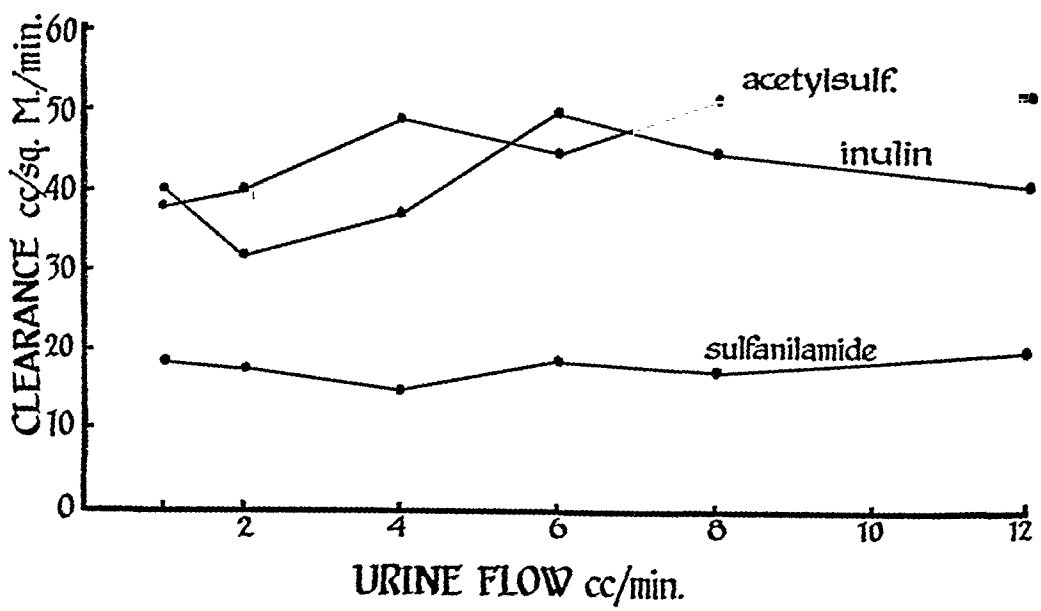

Fig. 1. Relation between clearance and urine flow. Each point represents the mean of from 5 to 25 clearance determinations.

acetylsulfanilamide were, respectively, 47.61, 19.32 and 48.21 cc. per minute per square meter of body surface area.

DISCUSSION. Marshall and associates (1) have conducted experiments which indicate that sulfanilamide is filterable through the glomerular membrane and these experiments have been confirmed in this lavoratory. However there is some evidence that about 20 per cent of the sulfanilamide present in plasma is bound to the plasma proteins (6, 7). This might not be available for filtration through the glomerular membrane; however, the difference between the clearances of inulin (46.54) and sulfanilamide (19.06) is too great to be explained by the presence of a non-filterable fraction of such a magnitude. The clearance of inulin is generally accepted as a measure of the glomerular filtration rate. Since the clearance of sulfanilamide is only 0.45 times as great as that of inulin, it may be concluded that part of the sulfanilamide which appears in the glomeru-

lar filtrate is reabsorbed through the kidney tubule. Thus sulfanilamide may be said to be excreted by the kidney by glomerular filtration plus tubular reabsorption. These results are in accord with those obtained by Marshall and associates on dogs (1), by this laboratory on rabbits (2), and by Stewart and collaborators on human subjects (8). Since the clearance of this compound was found to be independent of its plasma concentration it may be concluded, and may be shown, that its rate of excretion increases with increased plasma concentration.

Most of the acetylsulfanilamide clearances are based on the determination of the compound formed in the body following the administration of sulfanilamide. However, in some cases acetylsulfanilamide was administered, and it was found

TABLE 1

Statistical results of simultaneous clearance determinations

COMPOUND	CLEARANCE PERIODS	SUBJECTS	MEAN CLEARANCE* AND P.E. OF MEAN	STANDARD DEVIATION
Inulin	96	25	46.54 ± 1.63	21.06
Sulfanilamide	82	21	19.06 ± 0.63	7.36
Acetylsulfanilamide	96	25	47.95 ± 1.88	24.35

* Mean clearance (UV/P) per square meter of body surface area.

TABLE 2

Comparison of simultaneous clearance determinations

COMPOUNDS COMPARED	NUMBER OF PERIODS	MEAN DIFFERENCE / P.E. OF DIFF.	RATIO OF MEANS
Sulfanilamide / Inulin	82	15.70	0.45
Acetylsulfanilamide / Inulin	96	0.57	1.03
Sulfanilamide / Acetylsulfanilamide	82	14.51	0.39

following the administration of this compound to some of the subjects that from 1 to 3 mgm. per cent of the free compound was present in the plasma. In these cases the clearances of either sulfanilamide or acetylsulfanilamide were essentially the same whether the determination was made on the pre-formed compound or on that formed in the body.

Experiments have been reported in a previous paper which indicate that acetylsulfanilamide will pass through the glomerular membrane (2). It has also been shown by both in vivo (9) and in vitro (10) experiments on rats and rabbits that sulfanilamide is not conjugated by the kidney of these animals. Only inferential evidence is yet available to indicate that the human kidney is not able to conjugate sulfanilamide.

Evidence was obtained in this study which indicates that sulfanilamide is not

conjugated by the human kidney as it is being excreted. In two of the subjects only traces of the free compound were found in the blood following the administration of acetylsulfanilamide which was shown to be free of sulfanilamide. In these two subjects the clearance of acetylsulfanilamide was determined when sulfanilamide was almost completely absent. The clearances were then repeated on the same subjects on different days following the administration of sulfanilamide alone. The clearances of acetylsulfanilamide in these cases were found to be almost identical either in the presence or absence of sulfanilamide. These experiments indicate that sulfanilamide is not acetylated by the human kidney as it is being excreted, otherwise the clearance of acetylsulfanilamide would be expected to be greater in the presence of free sulfanilamide than in its absence.

The mean clearance of acetylsulfanilamide (47.95 ± 1.88) in the entire series was found to be almost identical to that of inulin (46.54 ± 1.63). The clearances of both compounds showed approximately the same variation. However, these variations were not parallel. The arithmetical average of the differences between the two clearances was 20.6 cc. per minute per square meter of body surface. Seven of the twenty-five subjects showed identical values by both tests. The cause of the rather marked differences in the clearances of the two compounds shown by the other subjects could not be determined. Since the acetylsulfanilamide clearance in some cases was higher and in others lower than that of inulin, no adequate physiological explanation can be given. It appears that other factors than glomerular filtration may have influenced the excretion, or the measurement of the excretion, of one or both of these compounds. The other possible physiological factors would be tubular excretion or tubular reabsorption of the compounds. The synthesis of acetylsulfanilamide by the kidney appears to be excluded by the data cited above.

SUMMARY AND CONCLUSIONS

1. Simultaneous clearance tests of sulfanilamide, acetylsulfanilamide and inulin were made on a series of unselected human subjects and the data were analysed statistically.

2. The mean clearance of sulfanilamide was found to be 0.45 times as great as the mean clearance of inulin in 82 determinations. This result was interpreted as meaning that sulfanilamide is excreted by the human kidney by glomerular filtration plus tubular reabsorption.

3. The mean clearance of acetylsulfanilamide was found to be almost identical with that of inulin in 95 determinations. However in individual cases the clearances of these two compounds were not always identical, but the degree of variations of these clearances were nearly the same.

4. The clearances of sulfanilamide, acetylsulfanilamide and inulin were found to be independent of the urine flow.

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THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 141 APRIL 1, 1944 No. 2

COMPOSITION OF THE BLOOD OF RABBITS IN GRAVITY SHOCK

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Received for publication October 26, 1943

The suspension of certain animals in the vertical position, with the head up, causes a decreased breathing rate followed by unconsciousness or fainting (syncope). This was demonstrated by Hall (1832) in dogs, and by Regnard (1868) and Salathé (1877) in rabbits. Hill (1895) studied the condition experimentally in dogs, cats, rabbits and monkeys, and found also a decreased blood pressure. He concluded that failure of the splanchnic vasomotor mechanism to compensate for the effect of gravity on the circulation caused "cerebral anemia or syncope or shock." More recently Mayerson (1942) has reported on similar results from the suspension of dogs and has referred to the conditions resulting from suspension as "gravity shock."

Studies have been made on the blood of rabbits before and during gravity shock to identify the physiological and chemical changes which occur in the animals, especially those in the blood. (Cf. preliminary report by Allison, Cole, Leathem, Nastuk and Anderson, 1943.)

MATERIAL AND METHODS. Common domestic rabbits of about the same age and size (from 2 to 3 kgm. in weight) were used. In the first series of experiments the blood of rabbits fed *ad libitum* on Purina rabbit chow was analyzed for various factors to establish "normal" or control values. One or two weeks later the same animals were suspended vertically, head up, without anesthesia, until they became unconscious. The period of suspension varied from 20 to 120 minutes, averaging 80, during which time regular breathing continued. The animals seldom struggled or showed signs of distress until just before becoming unconscious, when general contractions of skeletal muscles often occurred. Unconsciousness was recognized by the disappearance of all exterio-ceptive reflexes, by maximum dilatation of the iris and by relaxation of skeletal muscles. The rabbits were then returned to the horizontal position and the analyses were repeated. A second series of experiments was done similarly on rabbits from which all food had been withheld for 24 hours previous to the analyses. A third series was carried out on fed animals by analyzing the blood of a normal rabbit and then immediately subjecting the animal to gravity shock and re-

TABLE 1

Data—averages and limits—on blood and plasma of control rabbits and those in gravity shock

The averages are from 1 to 4 determinations on each animal. The number of individual animals in each group is given in the column "n". All animals were fed *ad libitum* on Purina rabbit chow.

FACTOR	FED ANIMALS				NOT FED FOR 24 HOURS			
	n	Control	n	Shocked	n	Control	n	Shocked
Rectal temp., °C.	28	39.6	24	40.3	11	39.1	11	39.5
Limits		39.1–40.3		38.7–41.8		38.8–39.5		38.4–41.3
Blood								
Hematocrit	29	42.2	24	43.7	11	38.6	11	42.3
Limits		35.7–48.1		33.6–58.0		34.2–41.6		35.1–51.4
RBC, mil./cmm.	26	5.71	23	5.84	10	5.39	6	6.07
Limits		4.6–6.9		4.7–7.6		4.8–6.2		5.2–6.9
WBC, thous./cmm.	26	8.90	23	8.47	10	8.34	6	6.13
Limits		5.2–21.4		4.7–16.2		4.8–18.2		5.2–8.9
Arterial O_2, vol. %	15	15.0	16	14.8	6	13.7	5	15.5
Limits		8.1–20.2		10.3–18.4		11.2–15.7		13.2–18.8
Venous O_2, vol. %	7	8.7	9	3.8	4	7.2	7	3.1
Limits		4.5–12.3		1.6–4.9		2.9–9.9		0.5–4.9
Arterial CO_2, vol. %	16	35.2	16	15.4	7	32.1	5	14.0
Limits		21.8–50.3		8.9–23.6		27.0–40.5		10.9–21.2
Venous CO_2, vol. %	7	47.2	11	30.1	5	43.9	7	27.0
Limits		37.9–59.5		20.7–40.3		37.6–50.6		23.4–32.3
pH	16	7.33	22	7.12	11	7.34	11	7.01
Limits		7.31–7.37		6.81–7.28		7.31–7.37		6.89–7.11
Lactic acid, mgm. %	17	58	21	175	11	43	11	116
Limits		31–94		77–282		11–64		54–248
Pyruvic acid, mgm. %	15	3.3	20	4.9	8	3.7	10	4.8
Limits		1.8–5.0		2.1–6.9		1.6–6.0		2.6–6.8
Total keto acids, mgm. %	5	3.4	10	5.0	6	3.7	7	5.0
Limits		2.5–4.8		2.8–6.7		1.9–6.0		3.0–6.9
Plasma								
Spec. gravity	30	1.02554	24	1.02577	11	1.02561	11	1.02522
Limits		223–302		232–292		223–285		221–286
Glucose, mgm. %	33	152	27	427	14	115	12	74
Limits		100–202		112–872		71–140		40–125

TABLE 1—*Concluded*

FACTOR	FED ANIMALS				NOT FED FOR 24 HOURS			
	n	Control	n	Shocked	n	Control	n	Shocked
Plasma—Continued								
Protein, gm. %	23	6.18	24	5.72	10	6.37	6	6.79
Limits		5.4–7.0		4.8–7.0		5.8–7.2		6.2–7.7
NPN, mgm. %	19	35.5	23	47.5	10	39.5	10	60.2
Limits		21.0–56.0		32.1–67.1		27.0–50.0		35–110
Na, mM/l	16	138	19	131	11	133	11	135
Limits		122–144		129–151		125–136		122–150
Ca, mM/l	19	3.84	20	3.90	11	3.39	11	3.19
Limits		2.6–5.0		2.8–5.6		3.0–3.9		2.6–3.9
K, mM/l	17	5.28	20	5.84	11	4.88	11	7.15
Limits		3.9–6.6		4.2–7.0		4.4–5.1		5.3–12.2
Cl, mM/l	30	104	24	96	11	105	11	104
Limits		84–110		78–110		92–112		92–111
PO_4, mM/l	8	1.86	8	4.51	4	1.95	4	3.89
Limits		1.06–2.32		1.68–7.93		1.84–2.06		2.58–5.29

peating the analyses. The results of the third and first series were combined, since they were the same. The factors studied were: respiratory rate, rectal temperature, hematocrit, red and white cell counts, pH of blood and the content of oxygen, carbon dioxide, glucose, lactic acid and keto acids in the blood; the specific gravity of plasma and the content of sodium, potassium, calcium, chloride, protein, non-protein nitrogen and inorganic phosphate in the plasma. In a few cases arterial blood pressure was measured in the femoral artery by means of the glass capsule manometer (Anderson, 1940–41).

Blood samples for all determinations except pH and blood gases were taken by right and left heart puncture with a syringe containing heparin. Without removing the needle from the heart a second sample was withdrawn into another syringe containing oil but no heparin, for determinations of pH, carbon dioxide and oxygen. Aliquots of the heparinized blood were transferred immediately to precipitating fluids for determinations of lactic and keto acids, and to appropriate vessels for determinations of hematocrit and cell counts. The remainder of the whole blood was centrifuged for several minutes and the plasma collected for the other analyses.

The hematocrit was measured according to the technique of Wintrobe (1929), and the cell counts were made according to Todd and Sanford (1943). The pH was measured with a Beckman pH meter using a glass electrode at 29° ± 1.0°C. The readings were corrected to 38°C., using an experimentally determined factor.

The correction was negligible below a pH of 7.2. Whole blood oxygen and carbon dioxide were analyzed in the manometric apparatus according to the methods of Van Slyke and Neill (1924); whole blood lactate by the colorimetric method of Mendel and Goldscheider (1925), or that of Barker and Summerson (1941); pyruvate and total keto acids by the method of Lu (1939) using larger samples as recommended by Bueding and Wortis (1940). The specific gravity of plasma was measured by the falling drop method of Barbour and Hamilton (1926); the plasma glucose by the method of Folin and Wu as modified by Andes and Northup (1938–39); plasma non-protein nitrogen and total nitrogen by the Pregl micro-Kjeldahl method; plasma calcium by the Clark and Collip (1925) modification of the Tisdall method; plasma potassium by the colorimetric method of Shohl and Bennett (1928); plasma sodium by the uranyl zinc acetate method of Butler and Tuthill (1931); plasma chloride by the mercurimetric method of Schales and Schales (1941); and plasma inorganic phosphorus according to the method of Fiske and Subbarow (1925). Bladder urine flow was measured by means of catheterization.

RESULTS. *General clinical symptoms.* Rabbits held in a vertical position, head up, without anesthesia became unconscious in from 20 to 120 minutes depending upon age, physiological condition and psychological state. Young, well nourished, healthy and calm rabbits were less susceptible to gravity shock than old, poorly nourished, unhealthy and excitable ones. Immediately after suspension the shallow rapid breathing of control animals became deeper and slower until a nearly constant rate of about 75 per minute was attained, continuing until unconsciousness. At that time the rate declined rapidly and breathing stopped entirely unless the animal was returned to the horizontal position. The blood pressure decreased gradually from around 100 to 30 mm. Hg. The size of the femoral vein was smaller, and the color lighter, than normal, indicating a decreased venous return. Rectal temperature increased from an average of 39.5 to 40.1°C. Urine flow was markedly reduced, being suppressed entirely in some animals. When the animals were returned to the horizontal position they immediately began to recover, as recognized by an increased breathing rate, and within 20 minutes they were similar to the control rabbits in posture, but appeared to be tired for an hour or more after that. About 30 per cent of them never recovered normal behavior and died from 2 to 24 hours after return to the horizontal position. Autopsies on the animals which died revealed some congestion of blood vessels in the viscera, slight enlargement of the adrenal glands and whole or part of the spleen, although no measurements were made. The brain was not examined. No edema was present nor were gross lesions seen.

Specific chemical changes. As shown in table 1 several marked changes appeared in the blood as a result of gravity shock. The most striking change, common to all the animals, was the fall in pH from an average of 7.33 to nearly 7.00. The majority of animals with a blood pH below 7.0 died soon after shock or within 24 hours afterward. This shift in the acid-base equilibrium is accompanied by a decrease in carbon dioxide and by increases in lactate, phosphate, pyruvate, potassium and non-protein nitrogen.

The only outstanding difference between the animals fed and those without food for 24 hours was in the concentration of the plasma glucose. During gravity shock, the average plasma glucose increased from 152 to 427 mgm. per cent in the fed animals, whereas it decreased from an average of 115 to 74 mgm. per cent in the starved rabbits.

The average oxygen content of all animals was not altered in the arterial blood (14.8 vol. per cent) but was reduced in the venous blood from 8.0 to 3.5 volumes per cent during gravity shock. Thus the difference between the oxygen content of arterial and venous blood was much greater in the animals in shock than in the controls. The average arterio-venous differences before and during shock were 6.4 and 11.7 respectively.

The carbon dioxide content was decreased in arterial and venous blood from 33.7 to 14.7 and from 45.6 to 28.6 volumes per cent respectively.

The lactate increased from 58 to 175 mgm. per cent in the fed animals and from 43 to 116 mgm. per cent in the starved animals. Pyruvate increased from about 3.5 to 4.8 mgm. per cent in both fed and starved animals. The total keto acids, expressed as pyruvate, showed the same concentrations as pyruvate, proving that no other acid of that type was present.

The non-protein nitrogen increased in both fed and starved animals from 35.5 to 47.5 and from 39.5 to 60.2 mgm. per cent respectively. The plasma inorganic phosphate increased from 1.86 to 4.51 and from 1.95 to 3.89 mM per liter in fed and starved animals respectively.

The plasma chloride of fed animals decreased from an average of 104 to 96 mM per liter and showed no change from normal in the starved animals. There were no significant changes in the sodium or calcium contents of plasma. The plasma potassium of fed and starved animals, however, increased from 5.3 to 5.8 and from 4.9 to 7.2 mM per liter respectively. The larger increase in the starved animals was probably correlated with a later stage of shock in those animals, since more of them showed a blood pH below 7.0 than did the fed animals.

There were no significant changes in the hematocrits, plasma protein concentrations, the red and the white cell counts, and the specific gravity of the plasma of the fed shocked animals, suggesting that neither hemodilution nor hemoconcentration occurred as a result of gravity shock. In the blood from the starved shocked animals there were slight increases in hematocrit and red cell count, and a decrease in white cell count, but the meaning of these changes is not clear. The correlation between plasma protein and specific gravity was low in the blood from rabbits in gravity shock, as reported earlier (Cole, Allison and Boyden, 1943).

Discussion. The rabbit when suspended vertically is unable to compensate adequately for the effect of gravity upon the circulating blood. An accumulation of blood in the posterior regions therefore occurs, followed by visceral congestion, decreased venous return to the heart and a drop in the blood pressure. Those conditions would be expected to cause a reduction in cardiac output, as was indicated by the increase in arterio-venous difference of oxygen content. There was no reason to assume increased oxygen consumption, since the increase

in temperature was minor and struggling was rare. There is thus established a peripheral circulatory deficiency which becomes an early cause of a series of disturbances in the physical and chemical processes of metabolism. Unless corrected, the disturbances will end in death. All of them are interrelated, and since they occur so rapidly, it is difficult to distinguish the causes from the effects. It may be safely assumed, however, that the slowed blood flow results in a decreased supply of oxygen to the tissues and in a decreased supply of blood to the kidneys. Changes in the concentrations of metabolites in the blood will then be the direct results of stagnant hypoxia and depressed urine excretion. Upon these general concepts and with the aid of theoretical implications the following working hypothesis covering the development of gravity shock in the rabbit is presented.

The stagnant hypoxia leads to a decreased utilization of oxygen by the tissues. Thus an acidosis of metabolic origin develops. Lactate and pyruvate, for example, parts of the carbohydrate metabolic cycle, accumulate in the blood, because the aerobic processes involved with their removal are depressed. The syntheses of liver glycogen from blood lactate and of muscle glycogen from blood glucose are therefore reduced. Glycogenolysis in the liver, primarily an anaerobic process, is not reduced, resulting in the hyperglycemia of fed rabbits in gravity shock. The hypoglycemia in starved animals results from a depleted store of liver glycogen. The concentration of plasma glucose during gravity shock, therefore, will be unchanged, increased or decreased from normal depending upon the amount of carbohydrate stores in the animal before shock develops.

The liberation of inorganic phosphate from organic sources in the tissues is accelerated by the increased hydrogen ion concentration. The lack of energy derived from aerobic processes depresses the resynthesis of inorganic phosphate into organic combination. Inorganic phosphate therefore diffuses into the blood at an increased rate. Its accumulation in the blood occurs because excretion by the kidney has decreased. (Cf. Guest and Rapoport, 1940-41.) Loss of phosphate from the carbohydrate cycle contributes to the eventual depletion of energy from carbohydrate stores. One of the results of the disturbances in carbohydrate metabolism is the entrance of proteins into the cycle. Non-protein nitrogen derivatives therefore increase in the blood, not only because of decreased urine formation but also because of abnormal utilization of proteins.

Unless the cycles of metabolic disturbances initiated by suspension are reversed, death will inevitably ensue. When the rabbits are returned to the horizontal position, the rate of circulation of blood is restored, blood pressure returns to normal and bladder urine flow is resumed. Provided irreversible damage to essential tissues has not yet occurred, such return allows complete recovery. The characteristic features of gravity shock—acidosis, phosphatemia, acapnia, increased lactate, pyruvate, potassium and non-protein nitrogen and decreased venous oxygen—gradually disappear. These eight factors can be used as criteria to determine the severity of gravity shock and to follow the course of recovery.

SUMMARY

1. Rabbits suspended head up, without anesthesia, became unconscious in from 20 to 120 minutes, even though breathing continued at a rate about one-half that of normal. Thirty per cent of the animals died within 24 hours although all external symptoms appeared normal.

2. Other changes resulting from suspension were: *a*, marked reduction of blood pressure; *b*, suppression of urine flow; *c*, metabolic acidosis (pH = 7.0); *d*, decreased blood carbon dioxide and venous oxygen; *e*, increased plasma lactate, phosphate, pyruvate, potassium and non-protein nitrogen; *f*, increased or decreased plasma glucose; *g*, decreased plasma chloride in well-fed animals.

3. There were no constant or significant changes in hematocrit, plasma specific gravity, protein, sodium or calcium or in the blood cell counts.

4. Hemoconcentration did not occur.

5. Suspension resulted in a peripheral circulatory deficiency leading to tissue hypoxia. Altered concentrations of certain blood metabolites occurred, which were useful in determining the severity of shock, and the course of recovery when the rabbits were returned to the horizontal position.

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COLD SWEATING IN MOTION SICKNESS

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Received for publication November 11, 1943

Cold sweating may be defined as the sweating which occurs without an adequate thermal stimulus. It is usually motivated by emotional, psychic or painful stimuli and has been investigated in the past mainly in relation with the psychogalvanic reflex (Darrow, 1934, 1937). Cold sweating also occurs as one of the symptoms of motion sickness but has been given only scant attention in the most complete descriptions of seasickness by Desnoes (1920), Bennett (1926), Maitland (1931), Hill (1936) and Brooks (1939). During investigations on experimental motion sickness with men as subjects, it was found that cold sweating was the most reliable and constant indication of the onset of motion sickness. In the present investigation cold sweating has been investigated in two ways. An electrical device has been used which measures the onset of sweating and a calcium chloride water absorption plaque has been used to measure the rate of sweating.

METHOD. Men between the ages of 20 and 30 who had passed the Army 64 physical examination were used. They were subjected to periodic motion which involved changing linear and centrifugal accelerations varying between 1.0 and 2.0 g (acceleration of gravity). Individuals susceptible to motion sickness usually exhibited symptoms of this condition within a 20 minute period. The subjects tested were fitted with forehead and arm electrodes made of tin. The arm electrode was large in comparison with the two forehead electrodes and was moistened with one per cent sodium chloride solution. The two forehead electrodes, approximately one square inch in area and placed on the forehead over the supraorbital ridge, were dry. The electrodes were connected into an electrical circuit with a small driving voltage and a galvanometer to indicate current. In this circuit there is a high electrical resistance at the dry electrode—skin surface on the forehead. When sweating starts the collection of sweat between electrode and skin reduces contact resistance and the current increases. By this method the start of sweating can be noted before there is any visible evidence of sweat on the skin. The increase of current is probably proportional to the rate of sweat secretion when the rate is small, but when sweating becomes profuse the contact space between electrode and skin becomes filled with fluid and the current reaches a maximum. When this occurs additional secretion of sweat has no effect on the galvanometer current. Hence, the galvanometer method is useful only to indicate the onset of sweating or to measure variations in the rate of sweating only when the rate is small. The galvanometric method fails when the skin is moistened by thermal sweating. This occurs with environmental temperatures above 30 to 35°C.

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To measure rate of sweating when the rate was high enough for sweating to be visible calcium chloride plaques were used. These consisted of a small metal container cylindrical in shape and 5 mm. in depth, open at one end and having an area of 3.9 sq. cm. They were filled with anhydrous calcium chloride and the open end was closed with lens paper sealed to the circular edge. They were weighed to the nearest 0.1 mgm. and stored in a dry weighing bottle. A dry plaque was placed on the forehead between the electrodes and held in place by the electrode headband. The duration time of sweating was determined from the onset as indicated electrically to the time of removal of the plaque

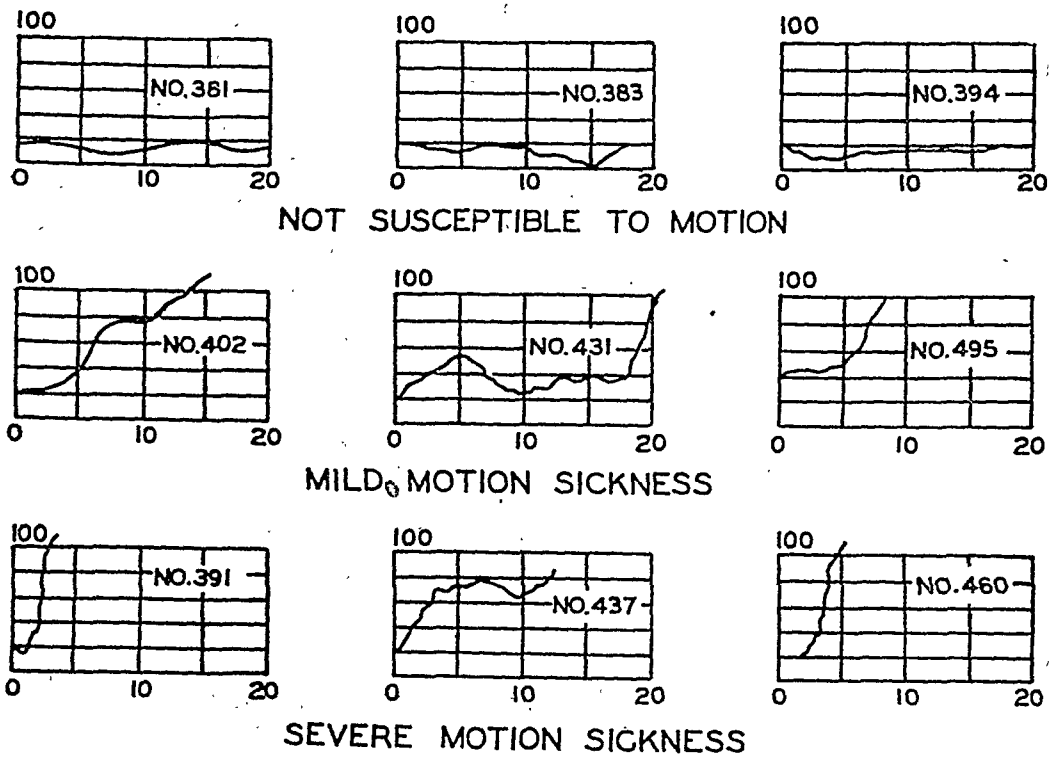

Fig. 1. Galvanometer records of onset of sweating. Abscissae, time in minutes; ordinates, galvanometer deflection in millimeters, 1 mm. is equal to 0.005 microampere.

which occurred as soon as the motion ceased. The sweating rate was calculated as milligrams of water per square centimeter of skin per minute.

Results. Figure 1 shows graphs of galvanometer deflections plotted against time in minutes. The three upper graphs are for control individuals who did not become sick and who did not sweat. The three middle graphs are for individuals who did not become sick to the extent of vomiting but who became nauseated and pale. The three lower graphs belong to individuals who vomited and exhibited the other symptoms of motion sickness as a result of less than 20 minutes' exposure to motion. The onset of sweating is quite distinct, and it is possible to predict oncoming sickness by the change in the galvanometer deflection before other signs are evident. In a few instances, approximately 5 to 10 per cent of individuals, sickness occurs without sweating. This is typical of the usual syndrome of motion sickness where one or more of the symptoms

of nausea, pallor, sweating or vomiting are likely to be absent or variable in intensity.

Table 1 contains data on temperature changes of the mouth and skin of the forehead as well as sweating rates in mgm. of water per sq. cm. per minute. It is to be noted that this is a true "cold sweating" in which sweating occurs with a decrease or no change in body temperatures. The drop in temperature was due to the increased cooling power of the environment caused by increased wind velocity due to motion. It is remarkable that in spite of this cold stimulus sweating was able to occur. The values in table 1 are representative results from more than a hundred tests.

TABLE 1

Rate of sweating (mgm. 1 min. cm.²), mouth and forehead temperature changes during the onset of motion sickness

	CONTROLS, NO SICKNESS			MOTION SICK						
NUMBER	665	700	799	664	681	701	757	795	836	850
Mouth temperature at beginning	37.6	37.2	36.1	37.2	37.5	36.8	37.2	36.4	36.5	37.1
Mouth temperature at end	37.3	36.8	36.1	37.2	37.2	36.6	36.0	35.6	36.3	36.3
Change in mouth temperature	−0.3	−0.4	0	0	−0.3	−0.2	−1.2	−1.8	−0.2	−0.8
Forehead temperature at beginning	31.4	29.0	26.2	35.4	30.0	31.8	24.6	28.6	25.8	34.8
Forehead temperature at end	30.4	27.4	24.0	31.4	27.8	29.0	22.2	24.8	24.6	34.2
Change in forehead temperature	−1.0	−1.6	−2.2	−4.0	−2.2	−2.8	−2.4	−3.8	−1.2	−4.6
Sweating rate, mg./sq. cm. min.	0.13	0.08	0.11	0.66	2.1	0.43	0.30	2.2	0.35	1.0
Environmental temperature	26	16	13	24.5	24	21	13	19	12	24

DISCUSSION. The phenomenon of cold sweating occurs during mental stress and during exposure to motion. During the onset of motion sickness in these experiments there was little or no mental stress such as fear, anxiety or apprehension. With the onset of nausea there was a certain amount of discomfort which may have caused cold sweating, or the cold sweating may have been a primary effect in itself and not caused secondarily by nausea or discomfort. Cannon (1939) states that cold sweating is part of the general preparedness reaction in which an animal secretes sweat for anticipated cooling for forthcoming muscular activity. This seems to be the only reasonable explanation for any usefulness which the reaction may have. As far as motion sickness is concerned, cold sweating is a useless and unnecessary reaction without any physiological value.

The symptoms of motion sickness are nausea, pallor, cold sweating, vomiting,

drowsiness, and a feeling of apathy, discomfort and depression. The motion sickness syndrome is probably a primitive defense mechanism in which the reaction to a harmful stimulus is emesis. A similar reaction results from other types of stress such as brain injury, pregnancy, digitalis (Hatcher and Weiss, 1922) and staphylococcus enterotoxin (Bayliss, 1940). In motion sickness it is a new environment involving motion to which the subject has not yet become conditioned. A variety of stimuli are effective in producing the characteristic train of symptoms but the motor mechanism is the same for all. Emesis resulting from a poisonous drug or from injury to the gastrointestinal tract is readily explainable as a logical defense reaction. But why emesis together with cold sweating results from motion or why it would be useful as a protective mechanism is not clear. An explanation, possibly on evolutionary development, is needed.

SUMMARY AND CONCLUSION

Cold sweating in man caused by motion involving changing linear and centrifugal accelerations has been studied by using a galvanometric device to indicate the onset of sweating. It has been found that the sweating occurs as a result of motion and when the mouth temperature is falling. There appears to be no useful physiological purpose in cold sweating and the mechanism is probably part of a primitive defense reaction.

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THE EFFECT OF VITAMINS OF THE B COMPLEX ON THE RESISTANCE OF THE ORGANISM TO ANOXIA[1,2]

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Received for publication November 30, 1943

Dietrich and Pendl (1) have shown that perfusion of the isolated frog heart with low oxygen concentrations causes a weakening of the contractions which are promptly restored by physiological oxygen pressure or by adding a minute quantity of riboflavin to the perfusion medium. In view of this finding it was thought worthwhile to study the influence of riboflavin on some other isolated tissue as well as on some of the effects of anoxia in the warm blooded intact animal. In addition some of the other vitamins of the B complex were studied in relation to their possible anti-anoxic effects.

It is known that anoxia depresses the reflex contraction of the digastric muscle (linguo-maxillary reflex) (2), the spontaneous rhythmic contractions of smooth muscle (3, 4), and cardiac contractions (1); it causes hypertrophy of the adrenal cortex (5, 6, 7, 8); decreases the critical fusion frequency (9); and diminishes or eliminates brain potentials (10, 11, 12, 13, 14, 15, 16, 17). Therefore, experiments were carried out on the linguo-maxillary reflex in the dog, isolated strips of smooth muscle, Straub preparations of the frog heart, the adrenal cortex in the rat and on the critical fusion frequency and electroencephalogram in man, in which the ability of the vitamins of the B complex to offset these effects of anoxia was studied.

METHODS. *Linguo-maxillary reflex (dog):* Acute experiments were performed upon 18 dogs weighing approximately 10 kgm. The animals were anesthetized with sodium amytal, 60 mgm./kgm., or with a combination of sodium amytal, 40 mgm./kgm., and chloralose, 20 mgm./kgm. Blood pressure was recorded from the femoral artery by means of a mercury manometer. The trachea was cannulated and connected to an electrical respiratory pump with adjustable stroke volume. Gas mixtures were inhaled from Douglas bags, filled by means of flow meters. Preparation of the digastric muscle and recording of its contractions were carried out as described by Blier and Kleitman (18). Condenser discharges at the rate of one per second were applied to the tongue in order to elicit the linguo-maxillary reflex. Denervation of the carotid sinus, when necessary, was accompanied by bilateral vagotomy. After the effect of anoxia on the reflex was determined the vitamin to be studied was injected intravenously into the femoral vein and the periods of anoxia repeated at 15 minute intervals. The

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[2] Aided by a grant from the Josiah Macy Foundation, administered by Prof. E. Gellhorn.

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effects before and after the administration of the vitamin were then compared. Riboflavin, thiamin, and nicotinamide were studied.

Isolated strip of smooth muscle (frog): A 5 mm. section of the frog's esophagus was removed, the wall cut through at one point and the strip suspended between two hooks and fastened to a lever so that the spontaneous contractions of the circular muscle could be recorded on a moving kymograph. The strip was kept in a bath of a phosphate, buffered Ringer's solution (pH 7.2) containing 0.5 per cent glucose, which could be perfused with either air or nitrogen. After a control experiment was performed in which the effect of nitrogen on the spontaneous muscular contractions was determined, air was allowed to replace the nitrogen, and the suppressed contractions returned to normal. Riboflavin in a concentration of 1–100,000 was then added to the Ringer's solution and the nitrogen perfusion was repeated 15 minutes later. The riboflavin was then removed and the experiment was repeated in riboflavin-free Ringer's solution for a second control.

Isolated heart (frog): Straub preparations of the frog heart were prepared and the heart perfused with a phosphate buffered Ringer's solution (pH 7.2) containing 0.5 per cent glucose through which could be bubbled either air or nitrogen. After a control record of cardiac contractions was made in the presence of air, the kymograph drum was stopped and the air was replaced by nitrogen. When a significant depression of the height of the cardiac contractions appeared, which usually occurred in from 3 to 5 minutes, the drum was again started and the vitamin to be tested was immediately added to the perfusion fluid. After five minutes, the nitrogen was replaced by air and the experiment terminated two to three minutes later.

Adrenal cortex (rat): Forty male rats weighing 200 to 250 grams were divided into five groups. The first group (9 animals) was used as a control group; the second group (9 animals) was not allowed to eat or drink for 48 hours; the third group (9 animals) was subjected to a barometric pressure of 294 mm. Hg (24,000 feet) for 48 hours, during which time the animals did not eat or drink; the fourth group (9 animals) was injected daily with riboflavin, 1 mgm. per 250 grams, for 6 days and then subjected to the same reduced pressure as group 3; and in the fifth group (4 animals) each rat received by stomach tube a daily dose for 6 days of liver concentrate plus thiamin, 1 mgm.; riboflavin, 333 micrograms; nicotinic acid, 5 mgm.; pyridoxine, 200 micrograms; and pantothenic acid, 250 micrograms. Then they were subjected to the same reduced pressure as groups 3 and 4. The animals of each group were then sacrificed and the adrenals removed and weighed.

Critical fusion frequency, electroencephalogram and pulse rate (man): In this group of experiments the effects of anoxia on the critical fusion frequency, electroencephalogram, and pulse rate of four males (medical students) were studied before and after the administration of relatively large doses of the vitamins of the B group.

For the determination of the critical fusion frequency (C. F. F.) a neon bulb whose flickering frequency could be controlled by an audiometer served as a source of flickering light. The area of the test field exposed to the eye equaled one degree, and when the flicker frequency was not being determined the test field

was covered by a screen. The subject observed the test field through an artificial pupil so as to avoid any variability resulting from pupillary diameter. Only one eye was used, the other being covered. At the appropriate signal the subject raised the screen and observed the test field while the experimenter gradually lowered the flicker frequency. When the first trace of flicker was observed the subject would indicate this by tapping the table and would promptly lower the screen. After a short practice it was possible for the subject to give very consistent results, individual readings varying by not more than one flash per second. The readings were always taken in groups of two or three and the results averaged.

During the intervals when the C. F. F. was not being measured, records were made of the electroencephalogram (E. E. G.) and the pulse rate. By means of bipolar leads the occipital alpha rhythm was recorded with the eyes closed. In addition, the effect of object fixation on the E. E. G. under conditions of anoxia was likewise studied. The pulse rate was recorded electrically with leads from the left forearm and leg.

The anoxia was produced by having the subjects breathe hypoxic gas mixtures (7 to 8.4 per cent oxygen) from Douglas bags through an oro-nasal oxygen mask. A system of two valves permitted the subject to inhale from the Douglas bags and exhale to the outside room air. The period of low oxygen inhalation was maintained until significant changes occurred in the C. F. F. and E. E. G. which usually required 10 to 15 minutes.

After the control effects were obtained the following amounts of vitamins were given to the subjects daily for a period of three weeks: thiamin, 6 mgm.; riboflavin, 12.6 mgm.; nicotinamide, 40 mgm.; pyridoxine, 0.8 mgm.; pantothenic acid, 2 mgm.; and also other factors from 12 grains of yeast.[4] Immediately after the period of vitamin administration the subjects were again tested.

RESULTS. *Linguo-maxillary reflex.* A series of control experiments was carried out in five dogs in which periods of anoxia were repeated at 15 minute intervals with no vitamins being given in order to determine the constancy of the depression of the reflex. The concentrations of oxygen used varied between 4.5 per cent and 9.1 per cent, and the duration of the inhalation of the anoxic mixture was determined by the response of the linguo-maxillary reflex. The degree and duration of the anoxic period was that necessary to produce a definite depression of the reflex. In all control experiments the results showed that the depression remained constant or became more severe and in no case did the anoxic effect become less.

Figure 1 illustrates this effect in a dog with carotid sinuses denervated and vagi cut.[5] This record shows that the repeated inhalation of 6.2 per cent oxygen for 2 minutes at 15 minute intervals resulted in a constant decrease of the reflex contraction. It is concluded from these results that any substance which is

[4] All of the vitamins used in the experiments reported in this paper were supplied by Abbott Laboratories.

[5] In some dogs it was necessary to denervate the chemoreceptor zones in order to obtain a depression of the reflex and then frequently a depression was not obtained. However, whenever an anoxic depression of the reflex was obtained, whether in the normal or "denervated" animal, it remained constant.

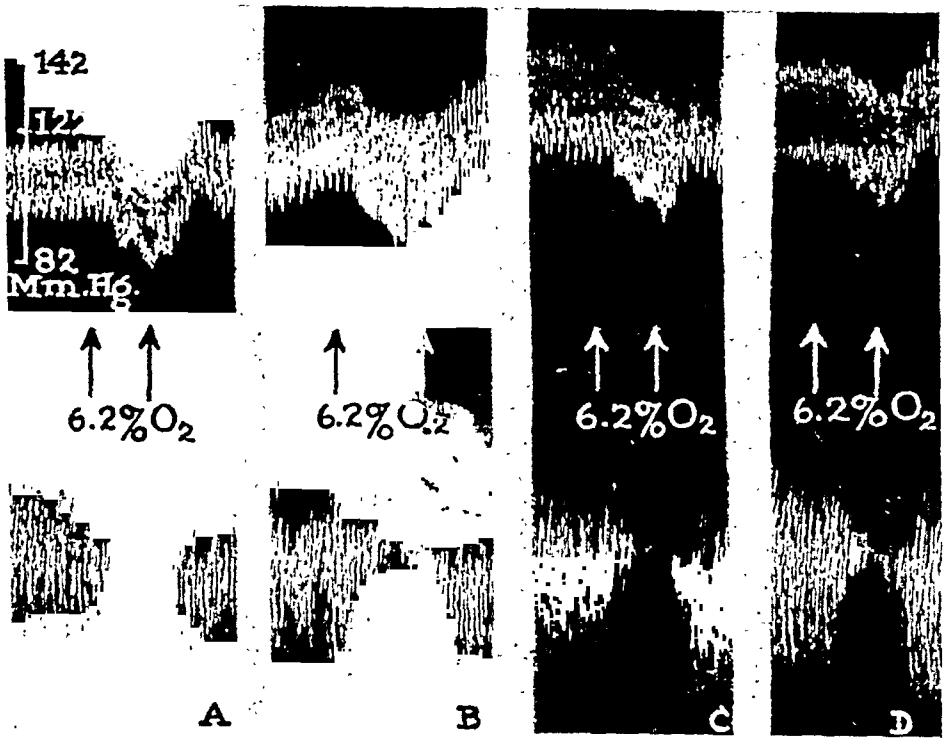

Fig. 1. The response of the linguo-maxillary reflex to repeated inhalations of anoxic gas mixtures. Dog, 10 kgm.; sodium amytal, 60 mgm. per kgm., intraperitoneally; blood pressure recorded from femoral artery. Artificial respiration. Carotid sinuses denervated and vagi sectioned bilaterally. Upper record, blood pressure; lower record, linguo-maxillary reflex. Duration of anoxia, 2 minutes. Parts A, B, C and D performed at 15 minute intervals.

TABLE 1

The anti-anoxic effect of riboflavin on the linguo-maxillary reflex

Dose = 1 mgm. riboflavin per kgm. I.V.

EXPERIMENT	NORMAL OR DENERVATED†	DEGREE OF ANOXIA	DURATION OF ANOXIA	HEIGHT OF REFLEX CONTRACTIONS DURING ANOXIA IN PER CENT OF CONTROL IN AIR				
				Before riboflavin	15 min. after riboflavin	30 min. after riboflavin	45 min. after riboflavin	60 min. after riboflavin
		%	*min.*					
1	Normal	6.2	2	0%	10%	40%	100%	
2	Normal	6.2	5	0%	0%	50%		33%
3	Normal	6.2	2	0%	35%	50%		
4	Normal	5.7	2	0% 88 sec.*	0% 63 sec.	0% 13 sec.	0% 13 sec.	0% 189+ sec.
5	Normal	5.7	2	0% 160+ sec.*	40%	0% 32 sec.	60%	100%
6	Denervated	6.2	2	50%	60%	10%	100%	100%
7	Denervated	4.5	3½	40%	40%	50%	100%	
8	Denervated	4.5	3½	40%	60%			100%
9	Denervated	6.2	4	30%	83%		62%	40%
10	Denervated	6.2	4	0%	0%	20%	50%	36%
11	Denervated	6.2	4	0%		0%	30%	50%
12	Denervated	4.5	2	0% 66 sec.*	0% 53 sec.	0% 50 sec.	0% 28 sec.	0% 7 sec.

* In these experiments the riboflavin did not prevent the abolishment of the reflex during anoxia but it did shorten the duration of the complete suppression as shown by the time in seconds.

† Bilateral carotid sinus denervation and vagotomy.

injected and followed by a decreased anoxic depression of the reflex has an anti-anoxic effect.

In a second group of 12 experiments performed on 7 dogs the effect of riboflavin on the anoxic depression of the linguo-maxillary reflex was studied. In all experiments riboflavin prevented to some degree the depression of the reflex by anoxia. Table 1 briefly summarizes this group of experiments.

Figure 2 shows the anti-anoxic effect of riboflavin on the linguo-maxillary reflex in the dog. Part A illustrates that inhalation of 6.2 per cent oxygen for two minutes caused a disappearance of the reflex which was restored upon the readmission of air. Immediately following part A, riboflavin (1 mgm./kgm.) was given intravenously, and 15 minutes later the inhalation of 6.2 per cent

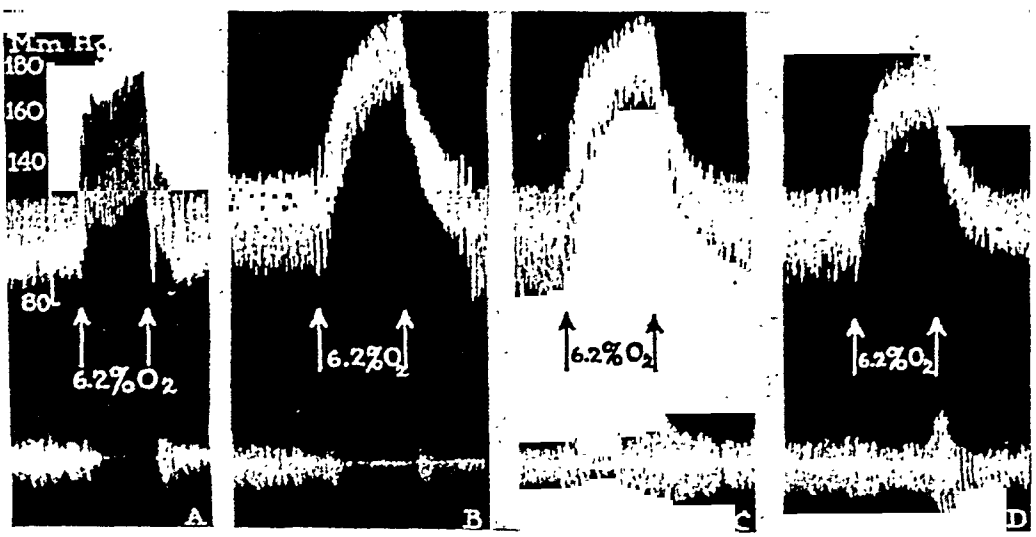

Fig. 2. The effect of riboflavin on the depression of the linguo-maxillary reflex due to anoxia. Dog, 9.1 kgm.; sodium amytal, 60 mgm. per kgm., intraperitoneally. Blood pressure recorded from the femoral artery. Artificial respiration. Upper record, blood pressure; lower record, linguo-maxillary reflex. Duration of anoxia, 2 minutes. Parts A, B, C and D performed at 15 minutes intervals. Riboflavin, 1 mgm./kgm. given intravenously between parts A and B.

oxygen was repeated (see part B). A very slight improvement resulting from the riboflavin is shown in part B, while part C, 30 minutes after injection, shows a definite improvement, and part D, 45 minutes after, indicates that the anoxic depression of the reflex has been completely abolished. In some experiments after the effect of an initial injection had disappeared a second injection of riboflavin again prevented the effects of anoxia on the reflex.

Hence from the figures and table cited above one may conclude that riboflavin alleviates the effect of anoxia on the linguo-maxillary reflex in the dog.

The same type of experiment as described for riboflavin was carried out using thiamin and nicotinamide. Three experiments on 3 different dogs were performed in which the dose of thiamin varied between 5 mgm. per kgm. and 25 mgm. per kgm. On three other dogs nicotinamide was used (10 mgm. per kgm.). In all 6 of these experiments neither thiamin nor nicotinamide showed any anti-anoxic effect.

Isolated strip of smooth muscle. In view of the fact that riboflavin prevented the effects of anoxia on the central nervous system as shown above and on cardiac muscle (1) and since these tissues are notorious in their susceptibility to oxygen lack, it was decided to test the anti-anoxic effect of riboflavin on some less specialized tissue cell which has a lower oxygen consumption. Therefore,

Fig. 3. The effect of riboflavin on the suppression of contractions of smooth muscle due to anoxia.

Part 1: Riboflavin-free Ringer's solution perfused with air. Part 2: Riboflavin-free Ringer's solution perfused with nitrogen for 25 minutes. Part 3: Riboflavin-free Ringer's solution perfused with air. Part 4: Ringer's solution plus riboflavin, 1–100,000 perfused with air. Part 5: Ringer's solution plus riboflavin, 1–100,000 perfused with nitrogen for 25 minutes. Part 6: Ringer's solution plus riboflavin, 1–100,000, perfused with air. Part 7: Riboflavin-free Ringer's solution perfused with air. Part 8: Riboflavin-free Ringer's solution perfused with nitrogen for 25 minutes. Part 9: Riboflavin-free Ringer's solution perfused with air.

experiments were performed on six preparations of isolated strips of smooth muscle. In each one of them riboflavin prevented to some degree the anoxic depression of the rhythmical muscular contractions. A typical experiment is illustrated in figure 3. Part 1 is a recording of the spontaneous contractions of an isolated strip of smooth muscle from the frog's esophagus in a bath of Ringer's solution perfused with air. Part 2 shows that after 25 minutes' perfusion with nitrogen, the contractions became irregular and were quickly restored by

air as shown in part 3. Between parts 3 and 4 riboflavin, 1–100,000, was added to the Ringer's solution and the preparation allowed to stand for 15 minutes. Part 4 is a recording in air, and in part 5 the perfusion with nitrogen was repeated. The record shows that in the presence of riboflavin, the perfusion with nitrogen suppressed contractions to a lesser degree than in the absence of riboflavin. Part 8 illustrates that when the Ringer's solution containing the riboflavin was replaced by riboflavin-free Ringer's solution, the nitrogen effect of part 2 returned. These experiments clearly show that in the presence of riboflavin, the anoxic depression of spontaneous smooth muscle contractions was considerably alleviated.

Isolated heart. The vitamins used were riboflavin, thiamin, thiamin pyrophosphate, and nicotinamide and the concentrations were varied between 1–10,000 and 1–200,000. Before testing for the anti-anoxic effect of a certain

TABLE 2

Effect of 48 hours' exposure to a barometric pressure of 294 mm. Hg on the adrenal weight of rats

Weights given in milligrams

RAT	CONTROL GROUP	NO FOOD OR WATER FOR 48 HRS.	294 MM. Hg 48 HRS.	294 MM. Hg 48 HRS. RIBOFLAVIN	294 MM. Hg 48 HRS. B COMPLEX
1	20	26	33	30	46
2	19	30	39	35	30
3	20	29	33	34	37
4	26	25	32	32	46
5	28	25	31	31	
6	24	21	39	39	
7	31	24	38	33	
8	25	23	33	38	
9	26	25	31	28	
Average.....	24.3	25.3	34.3	33.3	39.7

concentration of any vitamin, the concentration was first added to the preparation while perfused with air to determine whether or not any toxic effect would result. None of the above vitamins had any toxic effects when used in the above concentrations. In this group of experiments the vitamins of the B complex failed to show any ability to prevent the effects of anoxia on cardiac muscle.

Adrenal cortex. The results given in table 2 show primarily that neither riboflavin nor the entire B complex had any effects on the hypertrophy of the adrenal due to the anoxia. The group from which food and water were withheld for 48 hours showed no change from the control group in adrenal weight; thus, the increase in weight which occurred in the groups subjected to reduced barometric pressure was due to this latter factor and not to the fact that they did not eat or drink. The average weight of both adrenals for this group was 25.3 mgm. with variations between 21 and 30 mgm.

The third group which was subjected to a barometric pressure of 294 mm.

Hg for 48 hours showed an average weight of 34.3 mgm. with variations between 31 and 39 mgm., or a 36 per cent increase. The group which received riboflavin for 6 days prior to the 48 hour period of anoxia also showed an increase in adrenal weight comparable to group 3, the average being 33.3 mgm. with variations between 28 and 39 mgm. The group receiving the whole B complex had an average weight of 39.7 mgm. with variations between 30 and 46 mgm.

Critical fusion frequency, electroencephalogram and pulse rate. In a previous report (19) it was shown that under the conditions of these experiments there was a fall in critical fusion frequency, a rise in the pulse rate, a decrease in the effect of eye opening on the E. E. G., and the appearance of delta waves. The administration of large amounts of vitamins of the B group for 3 weeks and in one case for 5 weeks in no way offset these effects of anoxia. These results were obtained in four subjects (male medical students).

Discussion. Of the entire group of experiments described above, the most significant are those involving the linguo-maxillary reflex in the dog, which show that riboflavin prevents the depression effect of anoxia on reflex nervous activity. It is interesting to note that the maximal anti-anoxic effect of riboflavin did not occur until approximately 45 minutes following administration. Experiments carried out on the isolated strip of smooth muscle from the frog's esophagus also showed that riboflavin must be in contact with the tissue about 45 minutes before it is effected. It is suggested that the reason for this delay may be due to the fact that riboflavin must be first phosphorylated and combined with a protein molecule in order to be converted into a cellular respiratory enzyme. It has recently been shown that during anoxia a dephosphorylation of cocarboxylase takes place and that 30 to 60 minutes are required for its rephosphorylation (20).

The anti-anoxic effect of riboflavin on the linguo-maxillary reflex might be explained on the basis of an improved respiration or circulation. The respiratory factor is ruled out by the fact that respiration was controlled by a respiratory pump which provided a constant respiratory minute volume. The circulatory factor seemed a much more likely explanation, since Dietrich and Pendl (1) have shown that riboflavin improved cardiac contractions during anoxia in the isolated frog heart, and since in some of our experiments on dogs the administration of riboflavin was followed by an increased blood pressure response to anoxia (fig. 2). That an improved circulation is not the explanation is shown by the fact that the increased blood pressure response to anoxia is not correlated in time with the anti-anoxic effect on the reflex. Also, in animals whose vagi had been sectioned and carotid sinuses denervated and which in contrast to normally innervated dogs, showed a fall in blood pressure during anoxia (21), riboflavin retained its anti-anoxic effect. Further evidence which tends to substantiate the concept that the anti-anoxic effect of riboflavin is due to improved cellular oxidation in all the cells of the body and not to improved circulation is found in those experiments on the isolated strip of smooth muscle described above.

The dose of riboflavin used in these experiments on the dog appears quite large when compared to the daily requirement. Elvehjem and collaborators

(22) have stated that the daily requirement for riboflavin in the dog is 60 to 100 micrograms per kgm. per day. The dose used in the experiments reported here was approximately ten times this amount. This large dose was chosen purposely so as to saturate the tissues with riboflavin. However, this dose is far from toxic since Kuhn and Boulanger (23) have reported that the toxic dose of riboflavin is considerably greater than 1000 times the daily requirement.

The failure of the experiments reported here to confirm the investigations of Dietrich and Pendl, in which they showed the anti-anoxic effect of riboflavin on the frog heart, is rather difficult to explain since the studies on the linguo-maxillary reflex and strip of smooth muscle yielded positive results. One possible difference between their experiments and the ones reported here might have been that they may have used the phosphorylated riboflavin and failed to mention it. Therefore, several additional experiments were performed in which phosphorylated riboflavin was used. However, the phosphorylated compound also failed to prevent the effects of anoxia on the isolated frog heart; hence the inability to confirm Dietrich's results remains unexplained.

The inability of the vitamins of the B complex to prevent hypertrophy of the adrenal cortex under conditions of anoxia seemed rather surprising, especially since previous observations (24) showed that vitamin B deficiency led to adrenal hypertrophy, and that high vitamin B intake prevented adrenal hypertrophy resulting from exercise (25). Furthermore, it has been shown that lactoflavin, a member of the B complex, prevented the adrenal hypertrophy induced by thyroxin (26). It may be that the conditions of the experiment were too severe, since a hypertrophy of 36 per cent occurred in only 48 hours of anoxia. It would be interesting to repeat these experiments using a more mild anoxia over a longer period of time. Furthermore, since it has been shown that a dephosphorylation of cocarboxylase takes place under conditions of anoxia (20) and since it has also been demonstrated that adrenal cortical secretions are concerned with phosphorylation (27), it would be worthwhile to study the effects of giving large doses of the phosphorylated vitamins of the B complex on adrenal hypertrophy occurring under conditions of anoxia.

In addition to showing no anti-anoxic effects in man as measured by the critical fusion frequency and electroencephalogram, the vitamins of the B group did not raise the C. F. F. as previously reported by Simonson et al (28). This latter finding remains unexplained as approximately the same doses were used and were given for the same length of time.

SUMMARY

Experiments were carried out in order to determine whether or not the vitamins of the B group could increase the resistance of the organism to lack of oxygen.

1. *Linguo-maxillary reflex.* In a series of 12 experiments on 7 dogs it was found that riboflavin, 1 mgm. per kgm., given intravenously, prevented the depression of the linguo-maxillary reflex during the inhalation of low oxygen mixtures. The maximal effect occurred about 45 minutes after the administra-

tion and was found in normal animals as well as in those whose chemoreceptors had been eliminated. In an additional group of 6 dogs, thiamin, 5 mgm. per kgm. and nicotinamide, 10 mgm. per kgm. were found to be ineffective.

2. *Smooth muscle.* Riboflavin, 1–100,000, prevented the anoxic effects on the spontaneous rhythmical contractions of smooth muscle obtained from the frog's esophagus.

3. *Frog heart.* Riboflavin, phosphorylated riboflavin, thiamin, thiamin pyrophosphate, and nicotinamide in concentrations between 1–10,000 and 1–200,000 were found to be ineffective in alleviating the effects of anoxia on the isolated frog heart.

4. *Adrenal cortex.* The administration of large amounts of the vitamins of the B complex or riboflavin to male rats did not prevent hypertrophy of the adrenal cortex when the animals were subjected to 294 mm. Hg for 48 hours.

5. *Critical fusion frequency, electroencephalogram and pulse rate.* The daily oral administration of the vitamins of the B complex (thiamin, 6 mgm.; riboflavin, 12.6 mgm.; nicotinamide, 40 mgm.; pyridoxine, 0.8 mgm.; pantothenic acid, 2 mgm.; and also other factors from 12 grains of yeast) for a period of three weeks did not prevent changes due to anoxia in the critical fusion frequency electroencephalogram, and pulse rate.

Acknowledgment. The author wishes to express his appreciation to Prof. E. Gellhorn for his advice and interest in connection with these studies.

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THE DIURETIC ACTION OF THYROID IN DIABETES INSIPIDUS[1]

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Received for publication December 15, 1943

It is well established that the administration of thyroid affects the water exchange of dogs (1, 6, 21), cats (4), rats (3) and man (11). This hormone greatly exaggerates the polyuria of diabetes insipidus and, moreover, reveals cases of latent diabetes insipidus by enormously increasing water exchange (4); in normal animals it causes less alteration in urine volume. Brull (2) has shown that thyroxin has a direct renal effect: he transplanted kidneys from a dog pretreated with thyroxin to a normal dog, so that a comparison could be made of the urine flows from the thyroxinized and normal kidneys when both were supplied with the same blood at the same pressure. During a diuresis induced by saline infusions or removal of the pituitary, the urine flow was greater from the thyroxin treated kidney; but in the absence of measurements of renal blood flow, glomerular filtration and tubular reabsorption, the site of action of thyroxin within the kidney cannot be identified. In addition to this direct renal stimulation, thyroxin may affect urine formation through some extrarenal action, such as increasing cardiac output, which, by accelerating renal blood flow, might affect glomerular filtration.

The increased oxygen consumption of the hyperthyroid animal has been considered an extrarenal factor in the diuresis, despite the lack of immediate relationship between the two; but White, Heinbecker and Robinson (21) have found that although both thyroid and dinitroörthocresol increased oxygen consumption, only thyroid increased the water exchange in dogs with diabetes insipidus. Other dissimilarities in the responses to thyroid and to nitrophenols have been described by Tainter, Cutting and Hines (18), who stressed that protein wastage and high nitrogen excretion, so characteristic of hyperthyroidism, were not observed after the administration of dinitrophenol. Increased nitrogen excretion does cause an increased urine volume in diabetes insipidus, but according to Radcliffe (15), the elevation in urinary nitrogen in hyperthyroidism can hardly account for the added volume of urine excreted. The diuretic action of thyroid may be due in part to its stimulation of protein metabolism, but the experiments of White et al. (21) and of Bruhn (1) clearly show that an increase in oxygen consumption does not in itself cause a polyuria.

Since the processes immediately controlling urine flow are glomerular filtration and tubular reabsorption of water, the diuresis brought about by thyroxin must be due to changes in one or both of these. Pitts (14) has shown that the administration of thyroxin elevated the xylose clearance of normal dogs. While this clearance is not an exact measure of glomerular filtration, it provides good evi-

[1] A preliminary abstract of these experiments was published in Fed. Proc. 2: 38, 1943.

dence of an elevation in glomerular activity, and this glomerular effect has been confirmed by Heinbecker, Rolf and White (9), who used inulin clearances. We have reported increased creatinine clearances after feeding thyroid to dogs with diabetes insipidus (6) and suggested that this in part accounted for the diuresis. However, in dogs with latent diabetes insipidus, since there is an attendant decrease in the specific gravity of the urine, there must be some inhibition of water reabsorption in the renal tubules.

In the following experiments the effect of thyroid on glomerular filtration and tubular reabsorption has been studied in three groups of dogs: normal, with latent diabetes insipidus, and with severe diabetes insipidus. Complete destruction of the pars nervosa of the pituitary causes a severe and permanent polyuria; partial destruction results in a less complete deficiency of antidiuretic hormone and a water exchange which, under ordinary circumstances, is normal or only slightly increased. Under the stress of thyroid treatment, however, the dog with latent diabetes insipidus develops a severe polyuria.

Methods. Glomerular filtration was measured by creatinine clearances; tubular reabsorption of water by the creatinine U/P and by the chloride R/P (7). In the absence of pituitrin, chloride reabsorption is apparently normal, but because of a diminution in the reabsorption of water, the chloride is taken back into the blood in an abnormally high concentration. When the concentration of chloride in the plasma of dogs with diabetes insipidus was either lowered or raised by the injection of saline solutions, the concentration of chloride in the reabsorbate was always greater than in the plasma. The normal dog possesses an adaptive mechanism which regulates the concentration of chloride in the reabsorbate, so that when dilute saline or water is given the reabsorbate is more concentrated than plasma, while after hypertonic saline this relationship is reversed. Thyroid treatment does not affect this regulation in the normal dog, nor does it further disturb the already deranged mechanism in a dog with diabetes insipidus; however, it profoundly changes tubular reabsorption in a dog with a latent polyuria.

All experiments were performed on female dogs kept in metabolism cages. The procedures used in the collection and analyses of plasma and urine samples have been described (7). Inulin was determined by Harrison's (8) modification of the diphenylamine method, after all materials had been treated with yeast; urea by the manometric urease method of Van Slyke (13).

Hyperthyroidism was induced by adding five grams of dried thyroid gland[2] to the daily ration until the desired effect was obtained or until the dog began to lose its appetite. The antidiuretic hormone used was either Pitressin (Parke, Davis and Co.) or standard pituitary powder prepared according to Nelson and Munch (12).

Results. *Urine flow.* An increase in the water exchange was the invariable response to thyroid feeding, but the magnitude and the onset of the response were quite characteristic of the type of dog used (fig. 1). In severe diabetes insipidus,

[2] Mr. I. J. Klingaman of the Cudahy Packing Co. very generously supplied us with the dried thyroid gland used in these experiments.

the onset was rapid and the maximum diuresis attained within a few days; in latent polyuria, the onset was much more gradual, but if the medication was continued, the urine flow finally became as great as in severe diabetes insipidus. In the normal, the increase in water output was barely perceptible, and in no case was it more than fourfold.

Glomerular filtration. Creatinine clearance is the generally accepted measure of glomerular filtration in normal dogs; however, it was possible that during hyperthyroidism, when renal tubular reabsorption of water and of glucose were altered, creatinine excretion might also be affected. It seemed improbable, however, that thyroid would similarly initiate the renal tubular excretion of two such diverse substances as creatinine and inulin. Simultaneous clearances of the two substances were therefore run on three dogs, one from each group, during

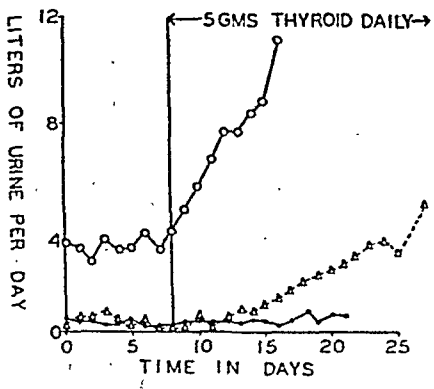

Fig. 1

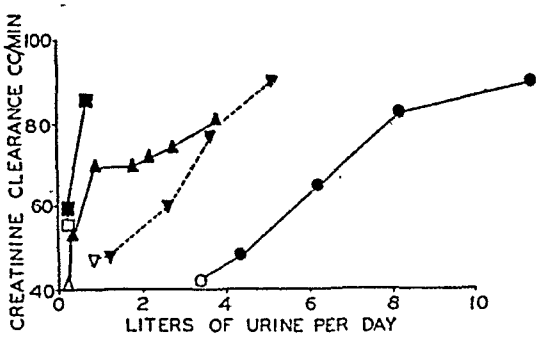

Fig. 2

Fig. 1. The effect of feeding thyroid on the urine excretion of a normal (solid circles), a latent polyuric (triangles) and a polyuric (open circles) dog.

Fig. 2. The effect of feeding thyroid on the urine volume and the glomerular filtration of a normal (squares), of two latent polyuric (triangles) and of a polyuric (circles) dog. The open figures represent basal values obtained before thyroid treatment; the solid figures data obtained during the onset of hyperthyroidism. Each symbol represents the average of at least three clearance periods. Glomerular filtration was measured at 2 to 3 day intervals in all except the normal dog; in this case there was an interval of two weeks between the second and third observations.

thyroid feeding, and the results differed only insignificantly, as may be seen in table 1. In all other experiments, glomerular filtration was therefore measured by the simpler creatinine clearances.

Glomerular filtration, in contrast to urine flow, was increased by about the same amount in all three groups (fig. 2). The daily urine volume of the dog with severe diabetes insipidus was tripled, while the increase in glomerular filtration is a little more than twofold. This suggests that the thyroid diuresis in diabetes insipidus is due almost entirely to a higher rate of glomerular filtration, and that the tubular reabsorption of water is affected to only a minor degree. However, in cases of latent polyuria, where urine flow is increased as much as forty times while glomerular filtration is approximately doubled, a greater part of the diuresis is due to a diminished reabsorption of water by the tubules. In one case, the glomerular and tubular effects on a dog with latent diabetes insipidus were tem-

porally dissociated, the creatinine clearance being almost doubled before any significant increase in urine flow occurred (fig. 2). In normal dogs the increased glomerular filtration only moderately affected the urine flow.

An increase in the rate of glomerular filtration could be accomplished by a greater production on the part of each active glomerulus, or by the recruitment

TABLE 1

Comparison of simultaneous creatinine and inulin clearances in hyperthyroid dogs

TYPE OF DOG	PERIOD	URINE FLOW	INULIN CLEARANCE	CREATININE CLEARANCE	INULIN CLEARANCE / CREATININE CLEARANCE
		cc./min.	*cc./min.*	*cc./min.*	
Normal	1	3.55	103	96	1.07
	2	2.80	98	93	1.05
	3	1.80	87	83	1.05
Latent diabetes insipidus	1	3.69	79	79	1.00
	2	3.23	83	84	0.99
Diabetes insipidus	1	7.25	91	93	0.98
	2	7.85	91	86	1.06
	3	8.21	95	92	1.03

TABLE 2

An experiment showing a parallel increase in glucose Tm and the creatinine clearance of a normal dog during the induction of hyperthyroidism

TREATMENT	PERIOD	DURATION	URINE FLOW	PLASMA GLUCOSE	URINE GLUCOSE	CREATININE CLEARANCE	GLUCOSE REABSORBED
		minutes	*cc./min.*	*mgm. per cent*	*mgm. per cent*	*cc./min.*	*mgm./min.*
Normal 11/20/42	1	20.0	2.25	445	2330	59	210
	2	20.1	2.94	447	2010	64	227
	3	19.5	3.06	452	2010	61	213
Hyperthyroid 11/27/42	1	23.2	1.93	464	5230	86	298
	2	21.5	2.60	456	4740	97	323
	3	19.1	3.27	452	3450	95	316
	4	19.2	4.68	440	2380	96	311
Hyperthyroid 12/4/42	1	19.9	7.26	658	3840	95	346
	2	22.2	7.22	726	4540	92	340
	3	16.8	8.27	788	4300	90	354

of quiescent nephrons. In the latter case there should be a proportionate rise in the capacity of the tubules to reabsorb glucose. Shannon, Farber and Troast (17) found the maximal rate of glucose reabsorption (glucose Tm) to remain constant in dogs during wide fluctuations in glomerular filtration induced by dietary changes. This indicates an extensive range of filtration rates by each

glomerulus and a constancy in the number of active renal units. In hyperthyroidism, however, the glucose Tm and the creatinine clearance show about the same percentile increase (table 2). This finding may have one of two interpretations: thyroid recruits nephrons or it stimulates the tubular mechanism of glucose transfer to about the same degree that it increases glomerular filtration.

TABLE 3

Comparison of the effect of thyroid feeding on the creatinine U/P in normal, polyuric and latent polyuric dogs

Each value is the average obtained from three 20-minute clearance periods

DAYS OF THYROID FEEDING	CREATININE U/P		
	Normal	Diabetes insipidus	Latent diabetes insipidus
0	284	21	131
2		23	113
4		11	141
7	126	19	125
9		12	35
11	219	17	42
14	87	16	50
18	135	18	18

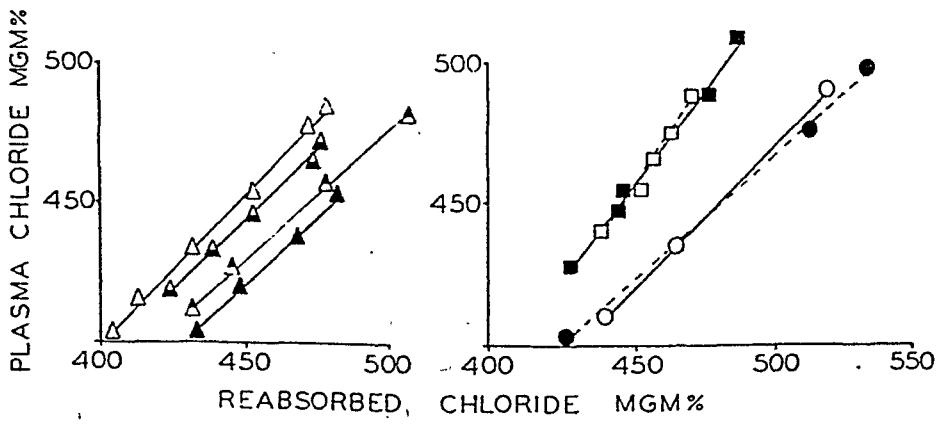

Fig. 3. The effect of thyroid feeding on the chloride R/P of a normal (squares), a latent polyuric (triangles) and a polyuric (circles) dog. In each experiment the plasma chloride concentration was elevated by the intravenous infusion of 2.0 per cent NaCl solution. The open figures represent basal values; the solid figures data obtained after three weeks of treatment with thyroid gland. The chloride R/P of the normal and of the polyuric dog is unaffected (shown on the right), but there is a progressive change in the dog with latent diabetes insipidus (shown on the left). The intermediate observations were made eleven (triangle with solid base) and eighteen (triangle with solid apex) days after thyroid was added to the diet.

Tubular reabsorption. The creatinine U/P provides information only of the tubular reabsorption of water. Tubular activity, as measured by this means at intervals of 3 to 5 days before and during thyroid administration, shows little change in the normal and polyuric dogs, but is strikingly decreased as the dog with latent diabetes insipidus becomes hyperthyroid (table 3).

Changes in tubular reabsorption, as measured by the chloride R/P, are also demonstrable only in dogs with a mild or latent polyuria (fig. 3). The progressive conversion of the renal response from normal to that characteristic of a dog without a source of pituitrin, suggests that thyroid feeding leads to an exhaustion of the already diminished supply of pituitrin, or that the renal tubules become refractory to its antidiuretic action. This second possibility was investigated in four dogs with severe diabetes insipidus, by measuring their responses to the same doses of pituitrin before thyroid treatment and at the height of hyperthyroidism. Dogs of this group were used because the renal response to an injected dose of pituitrin is not complicated by the action of an unmeasurable amount contributed to the circulation from an endogenous source. Since the antidiuretic effect was always less during hyperthyroidism, repeated observations were made during

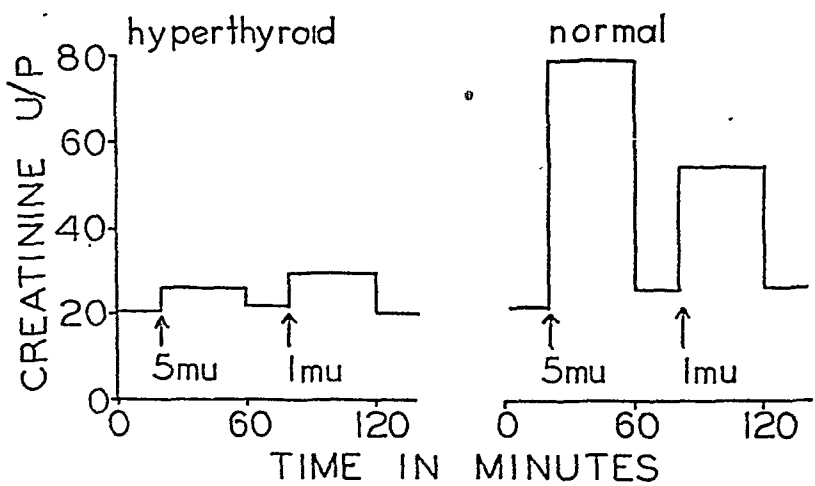

Fig. 4. Experiment showing the diminished effectiveness of pituitrin in the hyperthyroid dog. The subject, a dog with severe diabetes insipidus, was given one and five milliunits of pituitrin as indicated by the arrows. The first experiment was done while the dog was hyperthyroid; the second, two weeks later when the effect of the thyroid had subsided and the urine flow had returned to its basal level. The pituitrin was taken from a single ampoule.

recovery as the severe polyuria declined, to exclude deterioration of the pituitrin solution as the cause of the smaller responses in the hyperthyroid state. Test doses of pituitrin were injected at hourly intervals; the responses (fig. 4) were measured by the method of Hickey, Hare and Hare (10), who showed a linear relationship between the elevation of the creatinine U/P and the quantity of antidiuretic hormone injected into dogs with diabetes insipidus. In the experiment in figure 4 there is less difference between the normal and the hyperthyroid responses than in any of the other three cases, but the data from the two observations were considered more comparable as the basal creatinine U/Ps are almost the same. In other dogs, the threshold to Pitressin was raised, by prolonged thyroid feeding, from less than 0.5 to more than 20.0 milliunits. While this diminished response to pituitrin in the hyperthyroid dog is an adequate explana-

tion of the diuretic action of thyroid in dogs with latent diabetes insipidus, it does not exclude the possibility that the neurohypophysial remnant becomes depleted of pituitrin. There is indirect evidence that such exhaustion does not occur, but that, even in thyrotoxicosis, pituitrin may be released from the damaged pituitary. During the intravenous infusion of 2.0 per cent NaCl solution into a hyperthyroid dog with latent polyuria, the chloride R/P has fallen from 1.10 to 1.00 or less. A depression of the chloride R/P under these conditions is regularly seen in the normal, but never in the dogs with diabetes insipidus. According to Hare, Hare and Phillips (7) this signifies the action of pituitrin on the renal tubules.

Urea and salt excretion. Radcliffe (15) has shown that the increased water exchange in the hyperthyroid cat with diabetes insipidus is not due to an increased salt excretion. We have confirmed this in dogs. Fifty cubic centimeters

TABLE 4

Showing the independence of chloride excretion from chloride filtration in a normal dog before and after treatment with dried thyroid gland

PERIOD AND INFUSION		NORMAL						HYPERTHYROID					
		Duration	Urine flow	Creatinine clearance	Chloride			Duration	Urine flow	Creatinine clearance	Chloride		
					Plasma	Filtered	Excreted				Plasma	Filtered	Excreted
		min.	*cc./min.*	*cc./min.*	*mgm. per cent*	*mgm./min.*	*mgm./min.*	*min.*	*cc./min.*	*cc./min.*	*mgm. per cent*	*mgm./min.*	*mgm./min.*
	1	15.2	0.28	31	389	121	0.3	21.3	0.34	83	378	314	0.22
	2	15.8	0.38	40	390	151	0.45	13.5	0.54	90	378	340	0.12
1.5 per cent NaCl at 10 cc./min.	3	14.5	0.46	44	401	176	0.78	15.9	0.70	81	391	317	0.40
	4	14.7	0.44	44	415	182	2.55	17.0	1.00	90	409	368	2.94
	5	14.9	1.12	59	424	250	9.1	10.5	2.19	100	417	417	10.0
	6	14.8	0.88	54	416	224	8.7	15.9	1.85	88	422	371	9.7
	7	15.1	0.66	57	416	237	6.5	15.2	1.50	99	419	415	8.9

of 5.0 per cent NaCl were injected intravenously into untreated dogs, which were then returned to their cages where they had access to water. Urines, collected at regular intervals after the injection, were measured and analysed for chloride content. The procedures were repeated when the dogs were hyperthyroid. In every case the chloride excretion was practically unchanged, while the water excretion was greater.

This constancy of the chloride excretion is maintained despite the fact that the quantity delivered to the tubules by the glomeruli is increased. In table 4, the excretion of chloride by a normal dog receiving 1.5 per cent NaCl by intravenous infusion is almost exactly the same before and after thyroid treatment. Simultaneous creatinine clearances permit the calculation of the chloride filtered per minute, and, while this quantity differs by approximately 150 mgm. in comparable periods, the rate of excretion differs by a maximum of 2.4 mgm. per minute.

RECRUITMENT OF MAMMALIAN NERVE FIBERS

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Received for publication December 17, 1943

Gasser (1938) defined recruitment of nerve fibers as a systematic increase of the number of nerve fibers stimulated by the successive shocks of a submaximal train. This increase of the number of responding elements is shown by a corresponding increase of the amplitude of the spike potentials. The present study attempts to explain further the mechanism of recruitment.

METHOD. The nerve studied was the circulated peroneal in cats anesthetized with dial (Ciba, 0.7 cc. per kgm., intraperitoneally). It was cut centrally, at the hip, and peripherally, below the head of the fibula. Three pairs of chlorided silver electrodes were applied for stimulation. Two of these pairs were on the whole nerve, a few centimeters below the central cut; they will be referred to as the test electrodes. The other stimulating pair were placed peripherally on all the branches of the peroneal except the superficial. The latter branch was crushed distally and was placed on electrodes for recording purposes. More details about the preparation of the nerves and a diagram of the position of the several electrodes are given in a previous communication (Rosenblueth, 1943).

The stimuli were either condenser discharges or brief rectangular pulses with frequency controlled by thyratrons and an oscillator. Timed single pairs or trains of pairs of shocks could be delivered to the several stimulating electrodes. The detailed characteristics of the stimulators have been described elsewhere (Rosenblueth, 1943).

The nerve action potentials were led to a capacity-coupled amplifier and photographed from a cathode ray oscillograph.

Veratrine was injected into the aorta, below the inferior mesenteric artery. The leg on the recording side was fixed by means of drills, so that movements of the animal did not disturb the stimulating or recording electrodes.

RESULTS. A. *Recruitment after veratrine.* As observed by Gasser (1938), while recruitment was minimal or absent in normal mammalian nerves, it was prominent after treatment with appropriate doses of veratrine. For a given dose recruitment was prompter and proceeded to a higher level for faster than for slower frequencies of stimulation. It was possible to see marked increments of response with frequencies of 1 per sec. or slower.

As is true of other effects of veratrine upon nerve (see Acheson and Rosenblueth, 1941) the amount of veratrine necessary to produce a given degree of recruitment varied considerably from cat to cat. In a given animal the greater the dose of veratrine the more striking was the recruitment, up to the point where the nerve ceased to conduct.

Gasser (1938) found a correlation between the amplitude of the negative after-potential and the degree of recruitment; conditions which increased the after-

potential promoted the appearance of recruitment. The present observations do not support the inference of a strict correlation. Thus, with small doses of veratrine (e.g., 0.25 mgm. per kgm.) the negative after-potential was sometimes large while recruitment was minimal. Conversely, when several hours had elapsed after an injection of a large dose of veratrine (e.g., 3 mgm. per kgm.) the negative after-potential, initially large, had usually decreased in amplitude to only slightly greater than normal, while recruitment was still quite prominent.

With a given degree of veratrinization and a given frequency of stimulation, the rate of recruitment was more rapid and the final level of response was higher for relatively strong than for weak shocks. If the shocks had an appropriate intensity it was usually possible to reach a submaximal equilibrium after recruitment that did not change appreciably for several minutes.

In confirmation of Gasser's observations recruitment often took place when the intensity of the stimuli was such that no responses could be detected initially, even if the gain of the amplifier was high. It is likely, therefore, that recruitment may occur with shocks initially subthreshold for all the fibers in a nerve trunk. It is difficult, however, to exclude the possibility that one or a few fibers were activated from the start and that the highly shunted responses may not have been detected.

Within the range of frequencies of stimulation employed (1 to 200 per sec.) the stimuli could usually be weakened considerably after recruitment had developed, with slight or no decrease of the amplitude of the responses. Thus, it was possible for stimuli which were subliminal before recruitment to elicit practically maximal responses if at first stronger stimuli were applied, and later, after recruitment, the shocks were progressively made weaker without interrupting the train. A typical observation was the following. Veratrine (4 mgm. per kgm.) was injected and caused total block of the nerve for about 10 min. About 30 min. later practically all the A fibers in the nerve had recovered. Expressed in conventional units, the threshold was 20 and maximal responses required 28. If a series of stimuli at the rate of 30 per sec. was applied with an intensity of 22 to 25 the responses recruited after 1 to 3 min. to maximal. Further weakening caused a decrease of response, but about 50 per cent of the fibers were still active with a strength of 18, about 20 per cent with 17, and a few fibers still responded with an intensity of 16.

B. *The time course of the process which underlies recruitment after veratrine.* Two series of observations were made which bear on this problem. If the frequency of stimulation was too slow no recruitment took place (cf. Gasser, 1938). The critical frequency above which recruitment occurred was a measure of the duration of the process set up by each stimulus which might lead to an increase of the next response. This critical frequency varied, within a wide range, inversely as the dose of veratrine injected. Thus, after small doses, rates of stimulation of 30 per sec., or faster, were necessary to elicit recruitment, while after large doses it was often seen with rates of 1 per sec., or slower.

The independence of recruitment from the negative after-potential was emphasized by these observations. Thus, with moderate doses of veratrine (e.g.,

0.5 mgm. per kgm.) the negative after-potential usually lasted longer than 5 sec. whereas recruitment required frequencies of stimulation higher than 3 to 30 per sec.

In the second series of observations shocks were applied with a frequency which resulted in marked recruitment. The stimuli were then interrupted for variable periods. If these periods were relatively brief the responses remained large from the start upon the reapplication of the stimuli—i.e., recruitment had not subsided. As the rest periods were lengthened the responses obtained upon restimulation became progressively smaller, and with relatively long rest periods all signs of the previous recruitment disappeared.

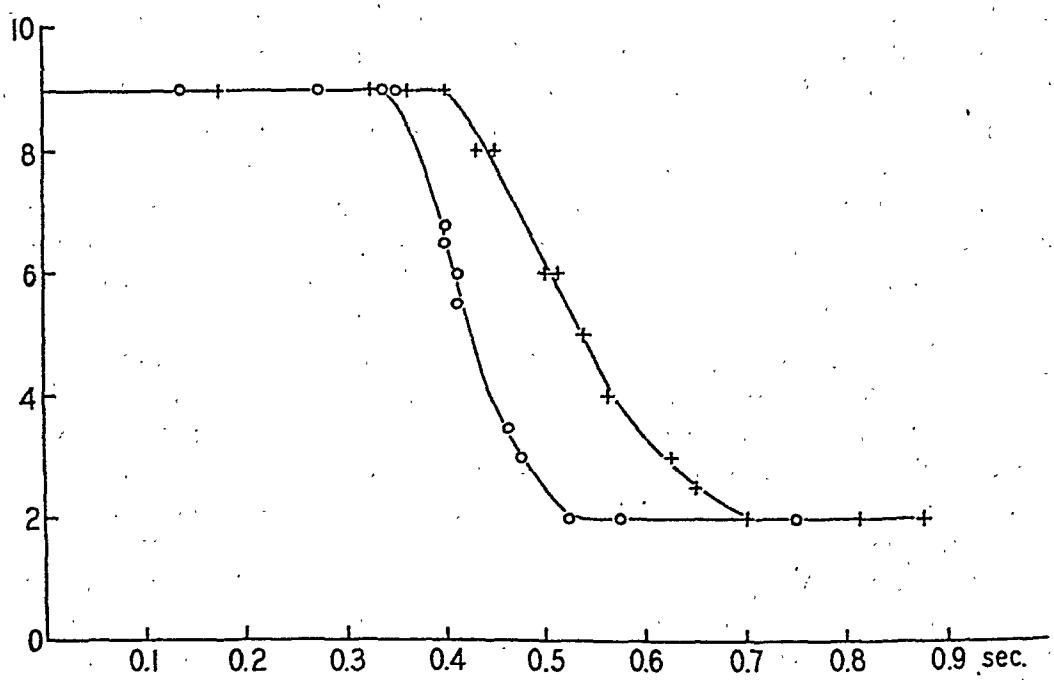

Fig. 1. Subsidence of the process underlying recruitment. Veratrine: 2 mgm. per kgm. The nerve was stimulated at a frequency of 20 per sec. (circles) or 50 per sec. (crosses). When recruitment had reached an equilibrium the stimuli were discontinued for a short period (abscissae, time in sec.) and were then reapplied. The ordinates correspond to the amplitude of the responses (in conventional units) upon renewal of stimulation. With short intervals (less than 0.35 sec.) of rest, the previous recruitment did not subside; with long intervals (more than 0.7 sec.) it subsided entirely.

The terms "relatively brief" and "relatively large" are used because the intervals necessary for partial or total subsidence of the recruiting process varied with the dose of veratrine, and the frequency and intensity of the stimuli. An increase of any of these three factors resulted in longer enduring recruitment. Of the three the most important was the amount of drug; the other two caused significant, but relatively small changes.

With fixed experimental variables the results were consistent. By plotting the amplitude of the response upon renewal of the stimuli against the interval of rest from stimulation curves were constructed which illustrate the time course of subsidence of the process responsible for recruitment. Figure 1 illustrates

two typical curves for stimulation with shocks of fixed intensity at frequencies of 20 and 50 per sec., respectively, in one preparation. The intensity was sufficient to insure maximal responses with both frequencies after recruitment. Similar curves were obtained when the responses were submaximal after recruitment.

The results agreed in the two series of observations. Thus, if a relatively high frequency was necessary for recruitment in given experimental conditions, then interruption of a train of stimuli for relatively short periods was sufficient for the disappearance of the pre-existing recruitment; conversely if recruitment took place with slow frequencies of stimulation then relatively long gaps in a train of rapid stimuli did not cause a decrease of response.

C. *Conditioning of recruitment by previous stimulation of the same fibers.* In a series of observations trains of just liminal stimuli were applied first to one pair of test electrodes and then, with a brief or no pause, to another pair. The

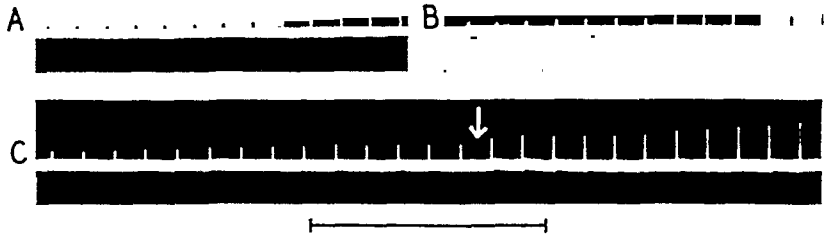

Fig. 2. The influence upon recruitment of changing the stimulating electrodes in the course of a train. Veratrine: 1 mgm. per kgm. A, recruitment upon stimulation of a central pair of electrodes; and B, upon stimulation of a more peripheral pair. C, stimulation first of the central pair, as in A, and, at arrow, shift to the peripheral pair, as in B. In this and the following figures, the records show the monophasic spike potentials of the A fibers of the peroneal nerve. The stimulus artifacts were usually too small for detection. Unless otherwise stated, the records begin with the first response to a train of stimuli, after a sufficient rest for return to basal conditions. The time calibration corresponds to 1 sec.

purpose of these observations was to elucidate the importance for recruitment of subthreshold local effects at the site of stimulation (see discussion). Typical records are reproduced in figure 2. A comparison of B with C shows that the amplitude and course of recruitment upon stimulation of the peripheral test electrodes was greater and faster, respectively, after stimulation through the more central electrodes than without this previous central stimulation.

In another series of experiments a frequency of stimulation (f) was determined which failed to produce recruitment when applied to either of the two pairs of test electrodes singly (fig. 3 A and B), but sufficiently rapid so that doubling the rate resulted in clear recruitment (fig. 3 C and D). If the initial frequency f was then applied to each of the two pairs of electrodes alternating the shocks, so that each site of stimulation was activated with a rate f but the nerve as a whole with a rate $2f$, then clear recruitment was seen again (fig. 3 E).

D. *Conditioning by stimulation of adjacent fibers.* In some experiments weak

stimuli were applied to the lower pair of test electrodes with a given frequency. After recruitment had reached a submaximal level of equilibrium a single maximal shock was applied to the upper pair of test electrodes, without interruption of the repetitive train. Figure 4 illustrates characteristic effects. After

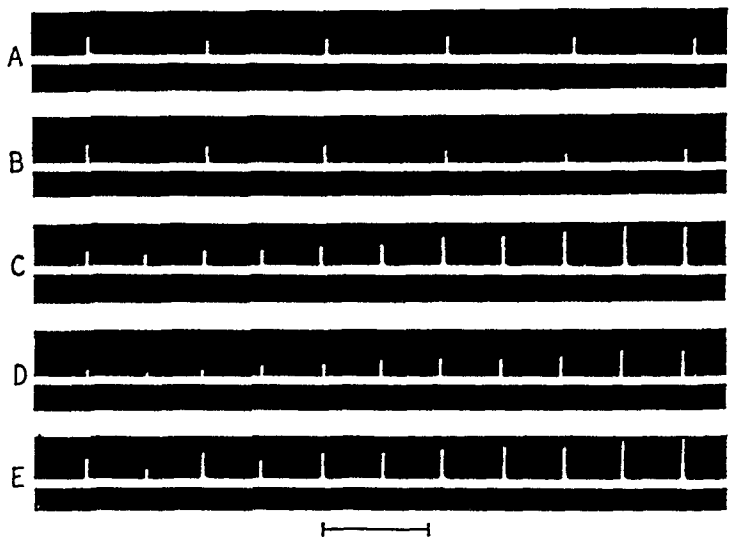

Fig. 3. Independence of recruitment from the site of stimulation. Veratrine: 1.5 mgm. per kgm. A and B, stimulation at a slow rate of a central and a peripheral pair of electrodes, respectively. The distance between the two stimulating cathodes was 2 cm. C and D, as in A and B, but with twice the rate. E, successive alternating stimulation of the central and the peripheral electrodes so that each pair delivered shocks with the same frequency as in A and B, but the nerve as a whole was stimulated at the rate used in C and D.

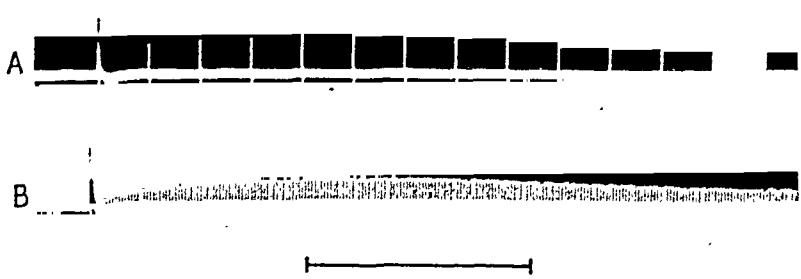

Fig. 4. Conditioning of recruitment by stimulation of additional fibers in a nerve. Veratrine: 1 mgm. per kgm. Continuous stimulation of a relatively peripheral pair of electrodes at the rate of 4.3 per sec. in A and 64 per sec. in B. The stimuli, quite weak, had been on for some time before the beginning of the records. The first large excursion is a maximal response to a single shock applied to a more central pair of electrodes, the following responses correspond to the continued stimulation of the lower pair.

the single maximal stimulus the responses first increased and usually later decreased to regain their original level after several seconds or minutes. As shown in the figure the frequency of test stimulation did not alter significantly the results. The reversible increase of response was accurately reduplicated if another conditioning stimulus was applied after a sufficiently long interval

(1 min. or more). If a conditioning shock was given before the effects of a previous stimulus had entirely subsided an increase was seen, but it was less striking and briefer than that elicited by the first conditioning shock (fig. 5).

In other observations the conditioning stimuli were applied to the branches of the peroneal not included in the recording electrodes. The procedure followed was to record the responses to a train of test stimuli without and with a previous conditioning shock. In figure 6 are reproduced typical results. It is

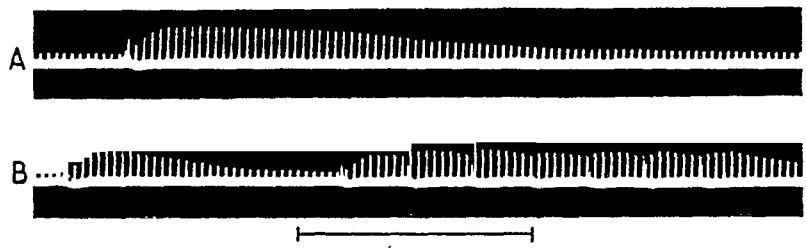

Fig. 5. As in figure 4, but showing the influence of repeated conditioning. In A are seen the effects of a first conditioning shock to the proximal pair of electrodes. An additional conditioning shock was given 5 sec. later. B shows the effects first of a 3rd, and then of a series of conditioning shocks delivered 10 sec. after the end of A.

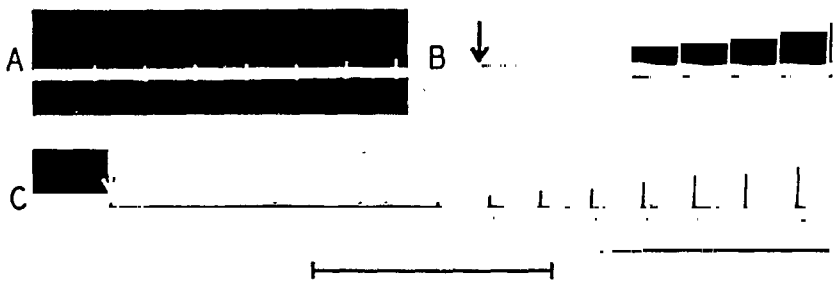

Fig. 6. Conditioning of recruitment by stimulation of adjacent, non-recording fibers. The test stimuli were applied to the sciatic and the record was from one of the branches of the peroneal. A shows unconditioned recruitment. B and C show the influence of a single maximal shock applied to the non-recording branches 0.64 and 1.35 sec., respectively, before starting a train of stimuli similar to that in A. The arrows indicate the application of the conditioning stimuli; the slight accident in the baseline is the stimulus artifact; there was no response of the recording fibers to conditioning stimuli even stronger than those used for the records.

clear that the rate of recruitment was markedly greater after conditioning, even if the conditioning shock preceded the test train by 1.35 seconds.

Discussion. Gasser (1938) considered two explanations of recruitment. The first was random fluctuation of thresholds. The shocks would begin stimulating the fibers when they happened to become hyperexcitable and then would hold them because succeeding shocks would fall in the supernormal phase of the preceding response. He gave arguments for dismissing fluctuation of thresholds as the only or main source of recruitment. The second explanation, which Gasser favored, was that subliminal excitation may sum over long intervals. The excitability curve following a subthreshold shock would be con-

tinued after the well known initial periods of latent addition and of postcathodal depression by a second summation period, variable in extent and duration. The process responsible for the second summation period would be localized to the region of the stimulating cathode and would run entirely subthreshold. In proof of the existence of this late period of increased excitability were the results of experiments with trains of subthreshold conditioning stimuli and with single submaximal test shocks, the cathodes for the stimuli from the two sources being superimposed. These experiments showed clearly that there was a late period of increased excitability.

A third possibility, not considered by Gasser, is that recruitment might also be due to the interaction of fibers adjacent in a nerve trunk. If impulses over some fibers should increase the excitability of neighboring fibers for a sufficiently long time, then recruitment would occur as a result of this increase. This prolonged enhancing interaction does in fact take place, especially after injections of veratrine (Rosenblueth, 1943).

The experiments illustrated in figure 2 show that recruitment at a lower pair of electrodes develops at a given time fully as well or better if a certain number of shocks has been administered previously to a different upper pair of electrodes instead of being applied to the same lower pair. This observation indicates that previous subliminal stimulation of a given region of the nerve is not indispensable for recruitment. In the record in figure 2 C, when the site of stimulation was shifted, although undoubtedly a good many of the previously responding fibers remained active, it is probable that some of them became silent and that other, previously inactive elements suddenly came into play. The expression "conditioning by previous stimulation of the same fibers," used in describing those experiments, is therefore only approximate.

The observations illustrated in figure 3 add further, stronger evidence in support of the view that interaction is the most important factor for recruitment after veratrine. At the slow frequency applied to each of the test electrodes in E the local effects were not sufficient to cause recruitment (A and B). With the faster frequency used in C and D recruitment took place, much as in E. It may be concluded, therefore, that the important factor for recruitment in these experimental conditions is not the rate at which any given point is stimulated, but the rate at which the nerve fibers respond—i.e., recruitment was brought out not by the stimuli, but by the responses.

Finally, the observations in figure 6 show conclusively the influence of interaction on the process of recruitment. The activity of some fibers promotes recruitment in other adjacent elements. This influence is not localized to any specific region, but is exerted all along the nerve.

The late increase of excitability responsible for recruitment may be long lasting (fig. 1). The horizontal, early portion of the curves in figure 1 need not be interpreted as showing that the underlying process is constant for a certain period and then rapidly subsides. The stimuli are probably supramaximal for the responding fibers during those early intervals. This interpretation is supported by the results described in p. 197, which show that once a fiber is recruited in the

course of a train of stimuli the shocks are indeed above the threshold of that fiber, since the stimuli may be weakened considerably without any decline of response. The decline of the process underlying recruitment probably begins much sooner than is shown by the curves in figure 1.

Evidence has been presented elsewhere (Rosenblueth, 1943) which indicates that the prolonged influence of activity in some fibers on the excitability of adjacent elements is exerted by a chemical factor, rather than by an electrical field—specifically, the release of K ions is suggested. The demarcation potential is often attributed to the difference of concentration of K ions inside and outside axons. It might appear then trivial to stress that interaction is chemical, rather than electrical, since changes in the external concentration of K would lead to changes of electrical potential. By a chemical action is meant here that it is not any electrical sign of the response of the conditioning fibers, but the liberation of a chemical agent, that modifies the excitability of neighboring elements. This chemical agent may well cause changes of the electrical equilibrium of the active and of the influenced elements.

There is no strict correlation between the changes of electrical response produced by veratrine and the appearance of recruitment (p. 197). Furthermore, the time course of the subsidence of the recruiting factor (fig. 1) is not similar to that of the regression of the negative after-potential. The data suggest, therefore, either that changes in the concentration of K ions are not the only source of potential differences in nerve, or else that some unknown chemical substance other than potassium may be responsible for the interaction which leads to recruitment. More evidence than that available is necessary to decide between these alternative explanations.

The experiments illustrated in figures 4 and 5 show that activity of previously inactive elements may lead to a reversible recruiting effect. The conditioning shocks elicit an increase of the test responses but this recruitment is followed later by a slow derecruitment to the initial level of equilibrium. The time course of the effect is not markedly different for different frequencies of test stimulation (cf. fig. 4, A and B). Obviously the important factor is the amount of chemical agent released by the conditioning stimulus. These results are comparable with those reported by Rosenblueth (1943), in which the conditioning stimulus was applied to non-recording, instead of to the tested elements, as was done here.

The results in figures 4 and 5, however, do not imply that the chemical agent released during the activity of a given fiber can influence only the electrical excitability of neighboring elements and cannot modify the excitability of the active fiber itself. Indeed, the action of the agent should be maximal on the fiber which releases it. This inference is supported by the supramaximality of the test shocks after recruitment, discussed above. The reversible effects of figure 4 are explained if it is assumed (see Rosenblueth, 1943) that the amounts of the chemical agent decrease with the successive discharges during repetitive activation (fig. 5). A large concentration of the agent caused by the large conditioning volley would then decrease in time to a lower relatively steady level.

If chemical interaction were the only factor which could cause recruitment the occurrence of the phenomenon would always depend on the activation of at least one nerve fiber by the first stimulus in the train. The observations of Gasser (1938), confirmed here (p. 197), of instances of recruitment in response to trains of stimuli initially subliminal for all the elements in the nerve trunk indicate, therefore, that the late period of local summation of shocks is also an effective mechanism for recruitment. It is inferred, therefore, that both mechanisms are at play in any given observation in which a fixed cathode is used for stimulation. When slow shocks are administered with a variable cathode (fig. 3), then only interaction can account for the increment of successive responses.

SUMMARY

Recruitment—a progressive increase of the number of nerve fibers stimulated by a train of submaximal test shocks—was studied in the circulated peroneal nerve of cats under dial anesthesia after injections of veratrine.

The process which underlies recruitment after veratrine can be long enduring (fig. 1). The time course of this process is not parallel with that of the negative afterpotential (p. 197).

Recruitment is uninfluenced by changes in the site of stimulation of a nerve in the course of a train of stimuli (figs. 2 and 3). It is elicited, therefore, by the responses, not necessarily by local effects of the stimuli.

Recruitment is promoted by single shock stimulation of the same (fig. 3) or other fibers in the nerve than those tested (figs. 4 to 6). The influence of the untested fibers shows the importance of interaction of the several elements in a nerve trunk for the development of recruitment.

It is suggested that recruitment is due to several factors; random changes of threshold (Gasser, 1938); local subthreshold changes of excitability (Gasser, 1938); and interaction exerted by the release of a chemical agent, probably K ions, by the active fibers (p. 202). After injections of veratrine the factor of interaction is the most prominent.

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THE GASTRIC EMPTYING TIME OF MAN AT HIGH AND NORMAL ENVIRONMENTAL TEMPERATURES

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Received for publication December 21, 1943

Decreased appetite is frequently observed in hot weather and there is a widespread belief that high environmental temperatures have a depressing effect on gastric activity. Some experimental evidence designed to explain a decreased appetite during hot weather has been presented (1, 2). The lack of appetite was interpreted as being correlated with an increased gastric emptying time. The present report is a comparative study of the gastric emptying time of 17 young men at temperatures of 77°F. and of 120°F.

METHODS. The 17 subjects were normal young men ranging in age from 18 to 28 years who were free from any observable gastro-intestinal abnormalities. None of the men were heat acclimatized at the time that the gastric emptying times were studied.

The subjects reported to the Laboratory in the morning without breakfast. After one-half hour of rest in the air-conditioned rooms a standard barium meal, consisting of 4 ounces of cooked oatmeal to which 2 teaspoons of sugar and 2 ounces of barium sulfate were added, was eaten as rapidly as possible. At exactly five minutes after starting the barium meal the first x-ray picture was taken. The second x-ray picture was taken at one hour and from then on the progress of the meal was followed by means of fluoroscopy at 15 minute intervals. X-ray pictures were taken whenever a decided change in size of the stomach shadow was observed. The final emptying time was confirmed by an x-ray picture. The x-rays and fluoroscopy were done in the standing position. Between observations the subjects remained seated.

This entire procedure for estimation of the gastric emptying time was determined on three occasions for each subject. The first time the temperature was 77°F., the second time 120°F. and the third time it was again 77°F.; the relative humidity was 50 per cent of saturation at both temperatures. An interval of 4 to 7 days separated each experiment.

The gastric shadows were traced onto paper from the developed x-ray films and the areas of the shadows were measured with a planimeter. All the gastric areas were expressed in per cent of the area at five minutes after eating the barium meal. The values for the two emptying times at 77°F. were averaged.

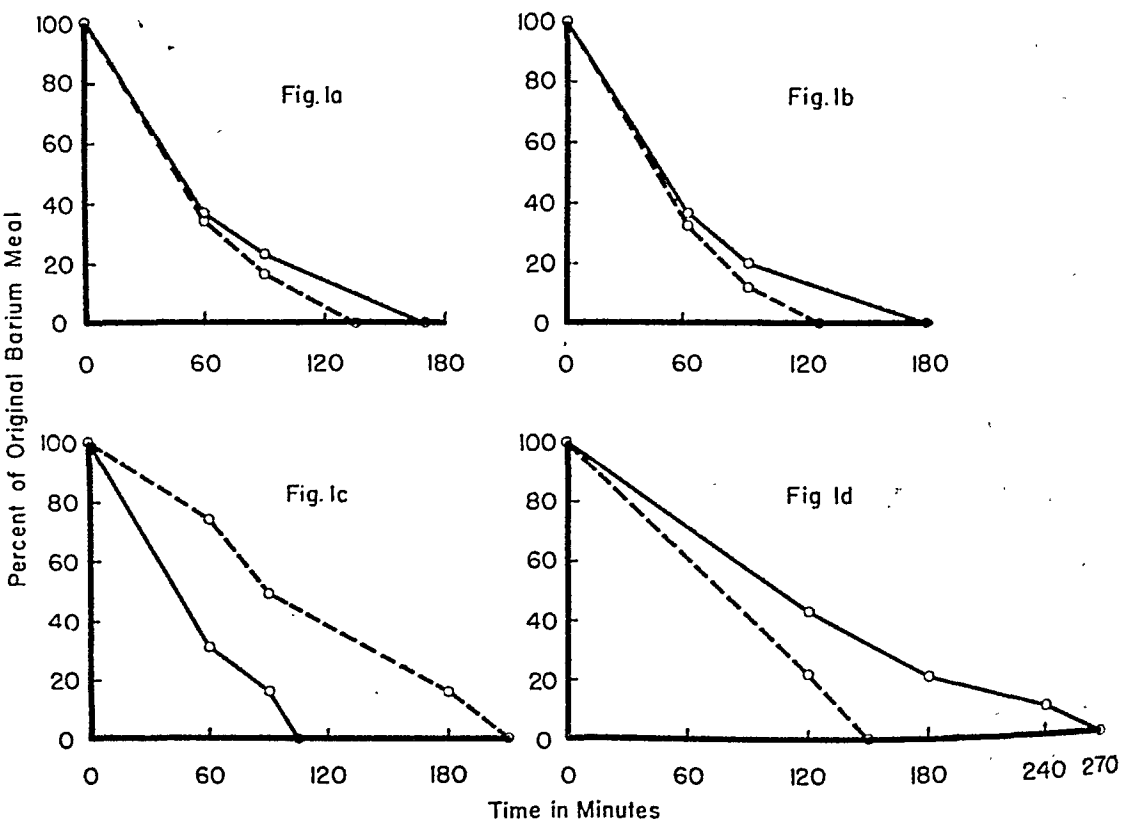
RESULTS. The major results are summarized in table 1. For analysis of the data the subjects were divided into four groups. Group I includes all 17 subjects, group II includes the 12 subjects who had a decreased gastric emptying time in the heat, group III includes the 4 subjects who had nearly comparable gastric emptying times in the heat and at normal temperatures and group IV includes the 1 subject who had an increased gastric emptying time in the heat.

A statistically significant decrease in gastric emptying time in the heat occurred (t-value for group I—13.3). Seventy-one per cent of the subjects had a markedly

TABLE 1

The means and the standard deviations of the final gastric emptying times at 77°F. and 120°F. for groups I, II, III and IV

TEMPERATURE	GROUP I		GROUP II		GROUP III		GROUP IV	
	77°F.	120°F.	77°F.	120°F.	77°F.	120°F.	77°F.	120°F.
Number subjects	17	17	12	12	4	4	1	1
Mean emptying time—minutes	170	136	178	125	163	150	105	210
S.D. ± minutes	55	47	52	39	62	61	—	—
Δ emptying time—minutes		−34		−53		−13		+105
Per cent Δ in emptying time		20		30		8		100



Graphs showing the rate of gastric emptying at 77°F. (solid lines), and at 120°F. (broken lines).

Fig. 1a. Average rate of gastric emptying for all subjects (group I).

Fig. 1b. Average rate of gastric emptying for the 12 subjects that had a marked reduction in emptying time in the heat (group II).

Fig. 1c. Subject No whose gastric emptying time was increased in the heat.

Fig. 1d. Subject Du who showed the greatest reduction in gastric emptying time in the heat.

decreased emptying time in the heat and 23 per cent had a small decrease in emptying time. Only one subject had an increased emptying time in the heat.

It is apparent that a decreased gastric emptying time is the usual response to the heat.

The acceleration of gastric motility in the heat is more pronounced in the later part of the gastric emptying phase. Figures 1a and 1b illustrate the fact that during the first hour the per cent of the barium meal that has left the stomach is only slightly greater at 120°F. than at 77°F.

The final gastric emptying time varies within wide limits in different individuals—from 105 to 270 minutes at 77°F. and from 90 to 240 minutes at 120°F. The means were 170 and 136 minutes respectively. The extremes of the effect of heat on the gastric emptying time are shown in figures 1c and 1d. Subject Du (fig. 1d) had a decrease in gastric emptying time of 120 minutes in the heat while subject No (fig. 1c) had an *increase* of 105 minutes in the heat.

Discussion. Meyer and Carlson (1) and Sleeth and Van Liere (2) observed that in the dog exposure to high environmental temperatures decreased hunger contractions and increased the gastric emptying time. They suggested a correlation between the decreased gastric motility in dogs and gastric derangements and loss of appetite in humans during heat waves. The apparent discrepancy in results obtained from dog and the present human experiments may be species differences. Differences in ability to regulate body temperature could not be the explanation. Neither the dogs nor the humans had more than a negligible increase in body temperatures.

Gastric tone and motility have been reported to be either increased or decreased by local applications of heat to the abdominal wall in dogs and humans (3, 4, 5). Raising the temperature of the normal human stomach by means of diathermy increases gastric motor function and decreases gastric emptying time (6, 7) but this is a different situation than the present or that in hot weather where the general body temperature is not appreciably elevated.

Decreased appetite and the desire for special foods during hot weather cannot be explained on the basis of decreased gastric motor function but may be related to decreased general activity. Whenever possible men tend to be less active when the environmental temperature is high. During the course of observations on about 100 young men who were working in temperatures of 120°F. in this Laboratory there was no striking lack of desire for food except in the men actually suffering from heat exhaustion or prostration.

Psychological and emotional factors associated with the unpleasantness of hot weather may influence appetite but they do not have any depressing action on gastric motility. The mechanism by which high environmental temperatures increase gastric activity is not known.

SUMMARY

1. Gastric emptying times were observed on 17 normal young men in rest at environmental temperatures of 77°F. and 120°F.

2. In all but one of the subjects the gastric emptying time was faster at the high temperatures. Twelve of the subjects had an average decrease of 30 per cent in the gastric emptying time when the temperature was 120°F.

3. Observations on about 100 normal men doing hard work at 120°F. failed to indicate any lack of appetite or any signs of decreased gastric activity except in actual heat exhaustion.

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THE SUCCESSFUL TREATMENT OF SO-CALLED "IRREVERSIBLE" SHOCK BY WHOLE BLOOD SUPPLEMENTED WITH SODIUM BICARBONATE AND GLUCOSE[1]

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Received for publication January 4, 1944

The point of departure for this work was our previous finding that lethal doses of certain barbiturates could be antidoted by the administration of sodium succinate (1). This was based upon the previous discovery of Quastel (2) that the barbiturates inhibited the oxidizing systems of the nervous tissues for glucose, lactate and pyruvate. Hence the succinate which was supplied represented utilizable substrate below the level at which metabolism was blocked, and sustained the life of the nervous system until the barbiturate was destroyed or excreted.

We hypothesized that the progressive tissue anoxia in shock might damage the oxidizing systems of the nervous tissues in a similar manner. When damage to the central nervous system and particularly to the midbrain had progressed to a sufficient degree, restoration of the blood volume and of the blood pressure would no longer lead to the permanent recovery of the respiratory and vasomotor centers. In other words the so-called "irreversible" stage of shock would have been reached. Under these circumstances it seemed possible that the more complex, less resistant and less abundant oxidation systems for glucose, lactate and pyruvate might be damaged before the system for succinate was involved. Sodium succinate might therefore be useful in providing sufficient energy for cell function and for the restoration of the damaged enzyme proteins.

The results of the present work show that the use of sodium succinate in conjunction with the restoration of blood volume brings about the recovery of a large proportion of animals from the so-called "irreversible" stage of hemorrhagic shock. However the results also indicate that the beneficial effect of the sodium succinate is due in large measure to the sodium ion. The succinic acid radical contributes somewhat to the therapy; but it is not specific, for it can be replaced by either lactate or glucose. This is in accord with the results of in vitro work, to be reported at a later date, which failed to indicate any impairment of the respiratory capacity of brain and cardiac tissue removed from animals in shock and at the point of death. The present work therefore emphasizes the rather forgotten factor of acidosis in shock and indicates that it may be important in determining the so-called "irreversibility" of far advanced shock.

[1] The work described in this paper was done under a contract recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Michael Reese Hospital, Chicago, Illinois (Responsible Investigator, Samuel Soskin).

[2] A part of the Committee on Shock of the Michael Reese Hospital.

METHODS. A series of 64 dogs was brought to the "irreversible" stage of shock by repeated bleeding. The animals were not anesthetized but were gentled by hand and voice and induced to lie quietly on animal boards, with some restraint by the use of ropes. The femoral vessels of both hind legs were exposed under local anesthesia (1 per cent procaine). One femoral artery was connected to a recording mercury manometer through a system filled with heparinized[3] saline.[4] The femoral artery and vein of the opposite limb were cannulated to serve for bleeding and reinfusion respectively.

The blood pressure was recorded continuously and determinations of the CO_2 capacity of the blood plasma were made at frequent intervals. The bleeding was done in stages as illustrated by the typical experiment in figure 1. The first bleeding amounted to roughly 20 per cent of the calculated blood volume on the basis that blood volume comprises 1/13 of the body weight (3). Fifteen to thirty

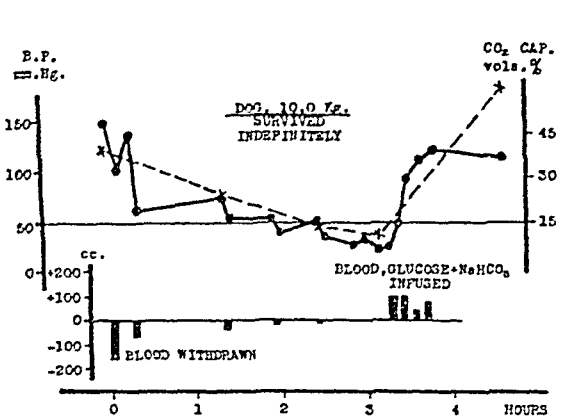

Fig. 1

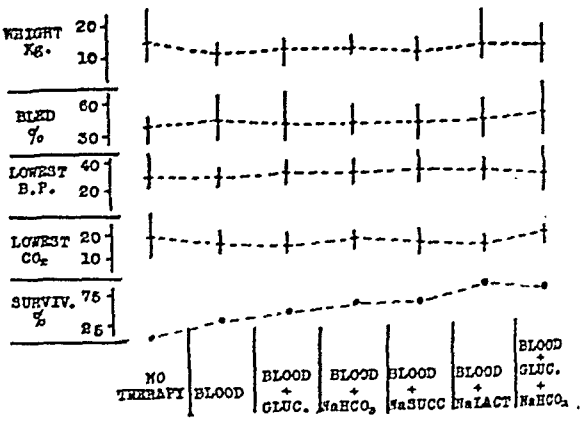

Fig. 2

Fig. 1. A typical experiment showing the amounts of blood withdrawn, the blood and supplements reinfused, and the time intervals involved. Note the parallelism between the changes in blood pressure and in the CO_2 capacity of the blood plasma.

Fig. 2. The range of values of certain critical data within each experimental group arranged so that the averages for the different groups can be compared. Note that all the average lines, except that for percentage survival, are essentially horizontal.

minutes later an additional 5 to 10 per cent of the calculated blood volume was removed. This usually reduced the blood pressure to below 80 mm. Hg. Further withdrawals of blood were guided by the reactions of the particular dog and were made in amounts and at intervals such as to reproduce a more or less standard pattern for all our animals. This pattern was the rapid reduction of the blood pressure to 50 mm. Hg or less and a slower decline over a period of $1\frac{1}{2}$ to 2 hours to the lowest pressures compatible with life. Therapy was with-

[3] The heparin preparation employed was "Liquaemin" generously supplied by Hoffman-La Roche, Inc.

[4] The avoidance of sodium citrate as an anticoagulant for this purpose is a matter of some practical importance. The fall in blood pressure as shock is induced allows the back-flow of a certain amount of the anticoagulant from the apparatus into the animal. Sodium citrate in such amounts and under such circumstances may contribute to the deterioration of the animal.

held until the CO_2 capacity of the blood plasma fell to 15 vol. per cent or less. In some cases this level was not quite reached but it was clinically obvious that the animal was at the point of death. These criteria more than fulfill the conditions of irreversibility reported by Wiggers (4) and by Necheles, Levinson et al. (5) (6). Our own control experiments (table 1, group 2) show that only 25 per cent of such animals recover even when reinfused with the total amount of whole blood which was withdrawn.

When the desired criteria for "irreversible" shock were obtained different groups of our animals were treated as listed below. Except for the first control group all animals were reinfused with the total amount of blood which had been withdrawn, minus the amount lost by sampling and by unavoidable waste. In the second control group the difference between the amount of blood withdrawn and that available for reinfusion (about 50 cc.) was replaced by physiological saline. In all the other groups this difference in volume was made up by solutions of the supplements noted. The point to be emphasized is that each animal, except those in the first control group, was reinfused with a volume of fluid (blood and supplement) equal to the total volume of blood which had been withdrawn.

1. First control group (nothing reinjected). 2. Second control group (whole blood). 3. Whole blood and sodium succinate (0.42 gram of the anhydrous salt per kgm.). 4. Whole blood and sodium lactate (0.58 gram per kgm.). 5. Whole blood and sodium bicarbonate[5] (0.43 gram per kgm.). 6. Whole blood and glucose (0.38 gram per kgm.). 7. Whole blood and glucose and sodium bicarbonate.[5] The amounts of Na^+ and of metabolizable substrate which were administered to the different groups were calculated to be equimolar. Heparin, in minimal amounts, was added to the blood as withdrawn. Pending its use later in the experiment, the blood was kept in a Pyrex flask in a refrigerator at 5°C. and warmed to body temperature just before reinfusion. This handling prevented hemolysis which would otherwise occur.

About one hour after the reinfusion of blood and other materials the cannulae were removed and the blood vessels tied. The wounds were packed with powdered sulphathiazol and sutured. The animals were returned to their quarters and allowed small amounts of drinking water at intervals during the evening of the experiment.[6] By the next morning those animals which survived seemed quite well and were placed on a free water and food intake. None of our animals developed significant infections of their wounds.

Throughout these experiments the animals either died within 18 hours after the institution of therapy or survived indefinitely. Most of the animals which died exhibited at post-mortem examination, the hemorrhagic state of the upper intestinal tract typical of far advanced shock. By indefinite survival we mean

[5] In order to avoid hemolysis the $NaHCO_3$ was not mixed with the blood to be reinfused but was administered separately in aqueous solution. The other materials were mixed with the blood just before reinfusion.

[6] If water ad lib. is allowed they tend to do themselves harm by overfilling the stomach, which results in vomiting.

that the animals were kept under observation for at least 10 days and at that time were outwardly perfectly well. There was one exception to this general rule. He lived for 4 days and was counted as a delayed death from shock.

RESULTS. The data for all our animals are summarized in table 1. It must be pointed out that the grouping of the results does not represent the order in which the particular animals were used. Every effort was made to alternate

TABLE 1

Effects of different treatments upon the survival of dogs brought to the so-called "irreversible" stage of hemorrhagic shock

(The experimental data given are the average values for each group.)

TREATMENT	NO. DIED	NO. SURVIVED	BLED PER CENT*	TIME IN SHOCK,** HRS.:MIN.	BLOOD PRESSURE, MM. Hg			PLASMA CO_2 CAPACITY VOLS. PER CENT			PER CENT SURVIVAL
					Control	Shock†	Therapy‡	Control	Shock†	Therapy‡	
1. Untreated control	8	0	37	3:38	145	28		37.2	18.7		0.0
2. Reinfusion of the withdrawn blood	6		41	3:38	136	27	64	41.8	16.7	26.1	
		2	44	4:23	130	28	108	35.6	14.5	27.9	25.0
3. Blood and sodium succinate	4		32	3:59	114	28	89	33.7	11.2	34.5	
		4	40	3:45	138	34	120	36.3	15.9	36.9	50.0
4. Blood and sodium lactate	2		26	2:45	165	30	115	28.9	9.7	31.5	
		6	42	4:49	134	29	121	37.1	12.4	40.1	75.0
5. Blood and glucose	5		52	4:54	135	27	82	35.6	12.3	21.4	
		3	27	4:38	127	32	105	32.8	14.1	22.1	37.5
6. Blood and sodium bicarbonate	4		28	5:24	125	31	74	35.9	16.8	64.8	
		4	44	4:12	131	26	113	33.3	15.0	68.8	50.0
7. Blood + glucose + sodium bicarbonate	6		34	3:10	131	22	87	43.4	15.7	51.4	
		10	47	3:32	128	25	107	41.2	15.8	62.1	62.5

* Indicates per cent of calculated blood volume, on the basis of 1/13 of total body weight.

** Indicates the time which elapsed from the first bleeding to the advent of the "irreversible" stage and the beginning of therapy.

† Recorded just before therapy was started.

‡ Recorded at least one hour after therapy was given, when dog was ready to return to animal quarters.

experimental animals from different groups in order to avoid the influence of seasonal, nutritional and other chance factors. The effectiveness of this procedure is indicated in figure 2, which shows the comparability of the various experimental groups as regards average weight, the proportion of blood withdrawn and the lowest levels of blood pressure and of plasma CO_2 capacity which were reached in the production of shock. It may be seen that while there was a

considerable range of each of these values within each group of animals, the lines drawn through the average values for each group are essentially horizontal. Only the graph representing percentage survival shows a real trend, and this is very significant.

Figure 3 graphically compares the results as regards survival in the different groups. It may be seen that all the animals which received no treatment died, and that only 2 of the 8 animals (25 per cent) to which all the withdrawn blood was restored were able to survive. When sodium succinate was added to the administered blood, 4 of the 8 animals (50 per cent) survived. As a test for the specificity of succinate a similar group of animals was treated with sodium lactate as the supplement. The results with lactate were as good or better than

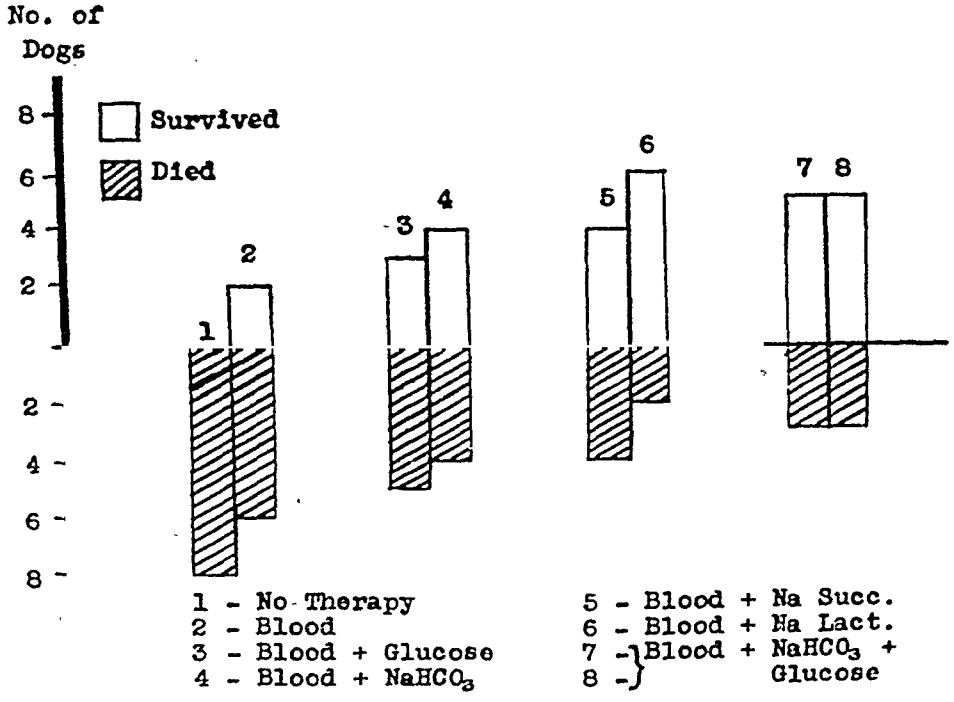

Fig. 3. Summary of results as regards number of dogs which survived or died in each experimental group. Each group consists of 8 dogs, and they are paired into larger groups of 16 according to the type of supplementary therapy.

with succinate, 6 out of 8 animals (75 per cent) surviving. In view of this lack of specificity it became of interest to test the Na^+ and the substrate components separately. When glucose was used as the substrate supplement, only 3 out of 8 animals (37.5 per cent) survived. In contrast to this the administration of sodium in the form of $NaHCO_3$ enabled 4 out of 8 animals (50 per cent) to survive. It was thus evident that the correction of the acidosis by the sodium component of the succinic and lactic acid salts was responsible for part of the benefit obtained. But it also appeared that additional metabolizable substrate probably contributed to the results. For these reasons a larger series of animals were tested by using both $NaHCO_3$ and glucose as supplements to the restored blood. Ten out of 16 animals or 62.5 per cent recovered from so-called "irreversible" shock.

DISCUSSION. Our results demonstrate that a large proportion of animals in so-called "irreversible" shock, at least of the hemorrhagic type, can be successfully treated. The necessary supplements to the restoration of blood volume are alkali and metabolizable substrate, and of those the former is probably of major importance.

Many years ago Yandell Henderson (7) first drew attention to the acidosis of shock. During the First World War, Cannon (8) (9) (10) elaborated on the subject. He pointed out the straight line relationship between the fall in blood pressure and the reduction in alkali reserve, as confirmed in our own work and illustrated in figure 1. He also emphasized the deleterious effects of acidosis upon the contractile power of the heart and the state of the capillaries. On the basis of these facts he was responsible for the use of $NaHCO_3$ solutions intravenously as a pre-operative measure in severely wounded men, and reported that this measure helped to maintain the blood and pulse pressures and in general led to favorable post-operative results.

The importance of acidosis in shock gradually came to be disregarded for several reasons. First, similar degrees of acidosis produced in normal animals by the administration of lactic acid were not accompanied by the criteria of shock. Second, McEllroy (11) reported that his shocked animals were not benefited by the administration of $NaHCO_3$ solutions. And third, $NaHCO_3$ was not tested as a supplement to whole blood or to plasma therapy.

To the above doubts as to the value of $NaHCO_3$ in shock it may now be replied that, even though acidosis is a result rather than a cause of shock, it is still an important complication and one which may make the difference between death and recovery when blood volume restoration is the only treatment. It should also be pointed out that McEllroy's conclusions were based largely on neurogenic shock and that in his shocked animals there was little or no acidosis. Finally $NaHCO_3$ solutions cannot be regarded as a substitute for whole blood or blood plasma and therefore cannot be expected to give beneficial results without them.

Our results hold promise of improved mortality statistics for the treatment of far advanced shock on the battlefield. But much remains to be done. Because whole blood is not readily available under those conditions, blood plasma supplemented with $NaHCO_3$ and glucose must be tested. The latter combination should also be tried on types of shock other than hemorrhagic. Finally the simplicity and abundance of the materials suggest the prophylactic use of a sweetened alkaline drink for injured men in early shock, for whom prompt blood volume replacement is not likely to be available.

SUMMARY AND CONCLUSION

1. Acidosis, while not a cause of shock, is an important factor in determining the reversibility of far advanced shock. Its relationship to shock was noted many years ago and then forgotten. More recently its value as a criterion of the severity of shock was emphasized by Necheles and Levinson (5) (12), and is confirmed by the present work.

2. In this work the criteria for the production of "irreversible" shock were: the rapid reduction of the blood pressure to 50 mm. Hg or less and a slower decline over a period of $1\frac{1}{2}$ to 2 hours to the lowest pressures compatible with life. Therapy was withheld until the CO_2 capacity of the blood plasma fell to 15 vol. per cent or less. In some cases this level was not quite reached but it was clinically obvious that the animal was at the point of death. One hundred per cent of such animals died when no treatment was given, and only 25 per cent survived when whole blood was used to replace the total amount bled.

3. Whole blood supplemented by $NaHCO_3$ and glucose resulted in the indefinite survival of 62.5 per cent of dogs brought to the stage of so-called "irreversible" shock.

NOTE

Since this paper went to press, we have come across a report (Coonse, G. K., P. S. Foisie, H. F. Robertson, and O. E. Aufranc. New England J. Med., **212**: 647, 1935) concerning the beneficial effects of alkali therapy in humans suffering from traumatic and slow hemorrhagic shock, as well as in experimental shock in dogs.

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THE ESTIMATION OF THE ANTI-FATTY LIVER FACTOR OF THE PANCREAS AND OF PANCREATIC JUICE BY THE USE OF THE COMPLETELY DEPANCREATIZED DOG MAINTAINED WITH INSULIN

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Received for publication December 24, 1943

The anti-fatty liver effect of pancreas or pancreatic juice has been determined in these laboratories in the following manner. Dogs delivered to the laboratory are carefully selected and only those used that are free of disease and are in possession of a vigorous appetite and a particular liking for raw meat. Such normal dogs are fed a diet high in protein (lean meat) and adequate in calories, vitamins and salts for a period of 2 to 4 weeks. The dogs are then completely depancreatized. After pancreatectomy each dog is fed twice daily (at 8:00 a.m. and at 4:00 p.m.) a mixture of 250 grams of lean meat, 50 grams of sucrose, 10 grams of bone ash and 1 gram of Cowgill's salt mixture (1). Three cubic centimeters of sardilene oil[1] and 5 grams of yeast[2] are fed with each morning meal. In addition, 125 grams of raw pancreas are added to each meal for the first 2 to 8 weeks after excision of the pancreas. Eight units of insulin are injected immediately after the ingestion of each meal. When the dogs have fully recovered from the operation and have regained a vigorous appetite, the raw pancreas is removed from the diet and the constituent to be tested is substituted. The feeding of the latter is then continued for a period of 16 to 20 weeks. The animals are then sacrificed, the entire liver excised, ground and thoroughly mixed, and a sample of it analyzed for total fatty acids (2). A normal fat content in the liver at the end of this 16 to 20 week period is taken as evidence of the presence of the anti-fatty liver factor in the constituent fed. It has been shown that a normal level of liver lipids can be maintained by feeding 1, 5 grams of raw raw pancreas (3); 2, 300 mgm. of choline chloride (4), and 3, 500 cc. of pancreatic juice (5). It must be realized, however, that these amounts may be more than the minimum amount necessary for maintenance of a normal liver lipid content.

A similar method has been used to determine the lipotropic effects of substances in dogs in which the external secretion of the pancreas has been completely excluded from the gastro-intestinal tract by ligation of all pancreatic ducts (4, 5).

The preliminary feeding of raw pancreas was adopted in order to ensure that, at the time the feeding of the constituent to be tested was begun, the dogs were

[1] Each cubic centimeter of sardilene contained not less than 100 A.O.A.C. chick units of vitamin D and 600 U.S.P. units of vitamin A.

[2] The yeast fed contained the following expressed in micrograms per gram: thiamine 20–150, riboflavin 30–50, nicotinic acid 340–930, pyridoxine 55, pantothenic acid 140–350, biotin 0.7–0.8, inositol 500, choline 4000–5000.

in good nutritional state, in possession of vigorous appetites and their livers free of abnormal amounts of fat. Dogs that showed a vigorous appetite at the end of the preliminary period of pancreas feeding usually retained a vigorous appetite throughout the subsequent period when raw pancreas was no longer included in the diet. All factors such as caloric intake, the protein intake, the vitamin and salt intake as well as the amounts of insulin injected daily were

TABLE 1

Effect of preliminary daily feeding of 250 grams of raw pancreas on the liver lipids of the completely depancreatized dog maintained with insulin

DOG NO.	BODY WEIGHT		PERIOD OF OBSERVATION AFTER PANCREATECTOMY			LIVER	
	Initial	Final	Total	Fed pancreas	Fed no pancreas	Weight	Total fatty acids*
	kgm.	*kgm.*	*weeks*	*weeks*	*weeks*	*gm.*	*per cent*
D1	13.9	13.5	4	4	none	433	3.1
D2	9.9	9.4	4	4	none	326	3.0
D3	13.7	13.5	4	4	none	401	3.8
D4	12.8	9.5	7	4	3	532	10.7
D5	9.2	7.6	8	5	3	330	16.2
D6	16.0	9.7	7	4	3	520	10.6
D7	12.6	9.5	6	3	3	300	18.3
D8	12.0	7.0	7	3	4	350	3.9
D9	13.0	8.7	8	4	4	280	14.8
D10	18.0	10.2	8	4	4	438	5.2
D11	11.5	8.4	11	3	8	570	21.0
D12	12.4	9.5	11	3	8	378	3.7
D13	9.6	7.8	15	4	11	281	29.9
D14	12.2	6.6	18	3	15	495	21.2
D15	14.2	9.2	18	3	15	512	24.6
D16	14.0	10.5	19	3	16	1116	17.3
D17	11.3	10.3	25	5	20	442	17.5
D18	12.0	11.7	24	4	20	1072	38.0
D19	12.2	7.3	24	4	20	1912	52.5
D20	9.0	7.9	24	8	16	472	19.8
D21	18.2	10.7	24	8	16	578	17.3
D22	13.3	9.0	24	8	16	523	21.5

* Total fatty acids are calculated on basis of wet weight of liver.

kept constant throughout the entire 16 to 20 weeks in which the constituent was being tested for its anti-fatty liver effect. Only in this way could the condition of the liver at the end of the 16 to 20 week test period be attributed to the presence or absence of a lipotropic factor in the constituent under examination.

The prolongation of the test period for 16 to 20 weeks is justified on the following experimental evidence. Although fatty livers have been observed as early as 3.5 weeks after pancreatectomy, their appearance at this short interval is not a constant finding (6). Livers containing in excess of 14 per cent fatty acids were found consistently only in those animals that had been maintained for 20 weeks or longer after pancreatectomy (6).

Despite the above mentioned precautions in the preparation of the test dogs, the procedure has been criticized on the ground that the addition of raw pancreas to the diet for the first 3 to 8 weeks after pancreatectomy may lead to storage of the anti-fatty liver factor of the pancreas (7–9). To answer this criticism, the present experiments were designed. It is shown here that preliminary feeding of as much as 250 grams of raw pancreas daily for the first 3 to 8 weeks after pancreatectomy led to no demonstrable storage of the anti-fatty liver factor of the pancreas, as measured by the development of fatty liver at various intervals after the feeding of raw pancreas had been discontinued.

Results. The livers of dogs D1, D2 and D3 (table 1) contained normal amounts of fatty acids. These dogs were sacrificed and their livers removed for lipid determinations 4 weeks after pancreatectomy; during this entire period of survival they received raw pancreas with their diets. These 3 animals serve as controls for the remainder of the dogs, since the fatty acid content of their livers is normal at the end of the preliminary period in which raw pancreas is fed.

Depancreatized dogs deprived of raw pancreas for 3 to 4 weeks. Four dogs (D4, D5, D6 and D7) were fed raw pancreas for the first 3 to 5 weeks after pancreatectomy; for the next 3 weeks it was excluded from the diet. The livers removed from these animals at the end of the 3 weeks contained 11 per cent or more of fatty acids.

Dogs D8, D9 and D10 were fed raw pancreas for the first 3 to 4 weeks after pancreatectomy and a diet containing none of it for the next 4 weeks. Only one of these dogs had a fatty liver.

Depancreatized dogs deprived of raw pancreas for 8 to 11 weeks. Dogs D11 and D12 were deprived of raw pancreas for 8 weeks after a preliminary feeding period of 3 weeks. A fatty liver was observed in only one of these animals.

The liver of a single dog (D13) was examined 15 weeks after pancreatectomy. It received raw pancreas for the first 4 weeks and none of it for the next 11 weeks. Its liver contained 30 per cent fatty acids.

Depancreatized dogs deprived of raw pancreas for 15 to 20 weeks. Nine dogs were deprived of raw pancreas for periods varying from 15 to 20 weeks. Six of them were fed raw pancreas for the first 3 to 5 weeks after pancreatectomy, and 3 of them received it for the first 8 weeks. Fatty livers were found in the livers of all 9 dogs.

Discussion. The rate at which fatty acids are deposited in the livers of completely depancreatized dogs maintained with insulin but at no time after pancreatectomy fed raw pancreas, has been measured by Kaplan and Chaikoff (6). In a study involving 30 dogs they showed that, although fatty livers may appear as early as 3 weeks after pancreatectomy, the presence of fatty livers at this early stage is not a constant finding. It was found that a period of *at least* 16 weeks was required to insure a constant finding of fatty acids in excess of 14 per cent in the livers of completely depancreatized dogs. In order to determine whether the preliminary feeding of raw pancreas for the first 3 to 8 weeks after pancreatectomy influences the rate at which fatty livers develop after the ingestion of the glandular tissue is discontinued, we need only compare

the above results with those obtained in the present investigation. The rate of accumulation of fatty acids in the livers as found in the present study does not differ significantly from that reported by Kaplan and Chaikoff (6). Thus it is shown in table 1 that a fatty liver may appear as early as 3 to 4 weeks after the last ingestion of raw pancreas, but its development at this early interval is not constant. On the other hand by the time 15 weeks or more had elapsed after the cessation of the feeding of raw pancreas, a fatty acid content of 17 per cent or more was found in all livers examined.

The lengthening of the preliminary feeding of raw pancreas to as long as 8 weeks (dogs D20, D21 and D22) did not prevent the deposition of as much as 20 per cent fatty acids by the time 16 weeks had elapsed after the last feeding of the raw glandular tissue. These results show that the rate at which fatty acids are deposited in the liver after cessation of raw-pancreas feeding is not influenced by the preliminary feeding of raw pancreas. They offer no support for the view that the anti-fatty liver factor contained in the fed pancreas is stored by the completely depancreatized dog maintained with insulin.

In previous work in which pancreatic juice was analyzed for the anti-fatty liver factor (4), the assay dogs were fed the raw pancreas in addition to the stock diet for the first 3 to 8 weeks after pancreatectomy. For the next 20 weeks the feeding of raw pancreas was discontinued, and the animals were fed pancreatic juice in addition to the stock diet. At the end of this 20-week period, the whole liver was removed and the lipid content measured. Three, 3, 6, 7 and 4 per cent fatty acids were found in the livers of 5 dogs that were so treated. Since these values are within the normal range, the conclusion was drawn that the anti-fatty liver factor is contained in the external secretion of the pancreas. This conclusion has been criticized (7–9) on the ground that the preliminary feeding of raw pancreas led to storage of the anti-fatty liver factor and accounted for the normal lipid levels observed in the livers of dogs fed pancreatic juice. The data presented here show conclusively that this is not true, since a preliminary feeding of raw pancreas does not alter the rate at which fatty livers develop after the withdrawal of the raw pancreas from the diet.

This investigation was aided by grants from Sharp and Dohme, Inc., and the Christine Breon Fund for Medical Research. The yeast was generously furnished by the Lederle Laboratories and the insulin by Eli Lilly and Company.

SUMMARY

1. For the first 3 to 8 weeks after pancreatectomy, dogs were maintained by daily injection of insulin and a diet containing raw pancreas in addition to lean meat, sucrose, salts and vitamins. The feeding of raw pancreas was then discontinued and thereafter the rate of deposition of fatty acids in the liver measured. The preliminary feeding period of raw pancreas did not influence the rate of deposition of fatty acids in the liver during the subsequent period when the feedings of raw pancreas were discontinued. From 17 to 53 per cent fatty acids were found in the livers of dogs examined at intervals of 15 to 20 weeks after discontinuation of the feeding of raw pancreas.

2. The relation of these findings to the assay of the anti-fatty-liver factor contained in the external secretion of the pancreas is pointed out.

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THE EFFECTIVENESS OF LIPOCAIC IN PREVENTING FATTY LIVERS IN COMPLETELY DEPANCREATIZED DOGS MAINTAINED WITH INSULIN

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Received for publication December 24, 1943

The presence of a factor in pancreas (other than insulin), the feeding of which prevents the development of fatty livers, has been repeatedly demonstrated (1–3). In 1936 Dragstedt and his co-workers prepared a fraction from pancreas which they called lipocaic and which they stated was effective in preventing and curing fatty livers in completely depancreatized dogs maintained with insulin (4). These workers put forth the claim that this factor is a second internal secretion of the pancreas (4).

It has been shown in several laboratories, however, that exclusion of the external secretion of the pancreas from the intestinal tract by ligation of the pancreatic ducts induces fatty livers (5–8). Moreover the daily administration of fresh pancreatic juice prevents fatty livers not only in dogs subjected to ligation of the pancreatic ducts but also in completely depancreatized dogs maintained with insulin (9). These observations offer no support for the view that the anti-fatty liver factor of the pancreas is an internal secretion; they show rather that the external secretion is the component of the pancreas involved in the anti-fatty liver effects and that the presence of pancreatic juice in the intestinal tract of the normal animal serves to maintain a normal lipid content in the liver. Best and Lucas in a recent review of this subject also reach the conclusion "that there is no evidence that the pancreas contains a second internal secretion" (8).

In support of the view that the pancreas contains a second internal secretion, Dragstedt and his co-workers offer their recent observation that the total loss of pancreatic juice from the body by complete pancreatic fistula does not produce fatty livers (10). But it is not necessary to invoke a second internal secretion to explain their failure to find fatty livers under these conditions. The type of fistula employed by these workers did not provide for complete loss of pancreatic juice from the body. They used a fistula in which a pocket of duodenum served to collect the pancreatic juice before it was eliminated. Absorption of pancreatic juice from this pocket of duodenum may be sufficient to account for the absence of fatty livers.

The method used in this laboratory to measure the anti-fatty liver effect of pancreas or a fraction of pancreas in the completely depancreatized dog maintained with insulin has been described in the preceding paper (11). Since lipocaic[1] has not yet been tested by this rigorous procedure, it seemed desirable to do so at the present time.

[1] The term "lipocaic" is reserved here for Dragstedt's fraction of pancreas.

EXPERIMENTAL. Fifteen adult dogs were used in this study. For 1 to 3 weeks before pancreatectomy, each dog was fed twice daily (at 8:00 a.m. and at 4:00 p.m.) a mixture containing 15 grams of lean meat and 3 grams of sucrose per kilo. Two grams of bone ash were added to each dietary mixture. Vitamin and salt supplements were added to the evening meal in the form of 3 cc. of sardilene oil, 5 grams of yeast and 2 grams of Cowgill's salt mixture (12). After pancreatectomy each dog received 250 grams of lean meat, 50 grams of sucrose and 125 grams of raw pancreas twice daily. Ten grams of bone ash were added to each dietary mixture. Salts and vitamins were fed in the manner described above. Eight units of insulin were injected immediately after each meal.

When the animals had fully recovered from the operation and had regained their appetites (2–4 wks.), the feeding of raw pancreas was discontinued. Lipocaic was then added to each dietary mixture in the form of a dry powder thoroughly mixed with the food. Lipocaic was kept in the ice box until used.

Blood samples were withdrawn from each dog during the control period prior to pancreatectomy as well as after pancreatectomy when they were receiving raw pancreas or lipocaic in their dietary mixture. Blood was removed from the animals while they were in the postabsorptive state. After receiving lipocaic for 8 to 20 weeks the dogs were sacrificed and their livers removed. The whole liver was thoroughly ground and mixed and samples taken for analysis. The oxidative procedures used for lipid analyses have been described elsewhere (13). Free cholesterol was measured in an acetone solution after the phospholipids had been precipitated.

RESULTS. I. *Liver Lipids.* 1. *Effect of feeding lipocaic derived from 15 grams of raw pancreas.* It has been shown elsewhere that the daily administration of a fraction derived from 5.5 grams of raw pancreas prevented the development of fatty livers in completely depancreatized dogs maintained with insulin (3). The effectiveness of this amount of pancreas was demonstrated for as long as 5 months; in dogs that had received 1 gram of AR (a dried defatted fraction of pancreas derived from 5.5 grams of the raw glandular tissue) for 5 months, the total fatty acid content of the liver did not exceed 4 per cent.

Six dogs were fed daily for 10 to 14 weeks an amount of lipocaic derived from 15 grams of pancreas and then sacrificed. The total fatty acid contents of their livers are recorded in table 1. In none of the 6 dogs examined did lipocaic prevent the development of fatty livers despite the fact that, as noted above, the amount of lipocaic fed was derived from an amount of pancreas that contained more than enough of the anti-fatty-liver factor necessary to maintain a normal lipid content in their livers. In 3 of the dogs the fatty acid content of the liver was over 40 per cent and in 5 of the dogs 25 per cent or over.

2. *Effect of feeding lipocaic derived from 100 grams of raw pancreas.* The dogs recorded in table 2 may be grouped into 1, those that received lipocaic for 8 to 9 weeks; 2, those that received it for 15 to 16 weeks, and 3, those that received it for 20 weeks. Two dogs (D7 and D8) were examined at intervals of 8 and 9 weeks after the commencement of the daily feeding of lipocaic equivalent to

100 grams of raw pancreas; the total fatty acid content of their livers was 8 and 19 per cent respectively. Two of the dogs that were fed lipocaic daily for 15 to 16 weeks had fatty livers; over 40 per cent fatty acids were found in the liver of dog D12. Massive infiltrations of fat were observed in the livers of

TABLE 1

The effect of feeding lipocaic derived from 15 grams of raw pancreas on liver fat of completely depancreatized dogs maintained with insulin

(Each dog received daily 0.35 gram of lipocaic. This amount was prepared from 15 grams of raw pancreas.)

DOG NO.	WEIGHT		PERIOD LIPOCAIC* FED	LIVER	
	Initial	Final		Weight	Total fatty acids
	kgm.	*kgm.*	*weeks*		
D1	10.0	6.0	10	489	50.2
D2	18.5	14.5	10	956	44.3
D3	9.0	7.4	10	312	7.8
D4	12.1	9.5	11	431	25.0
D5	11.0	7.7	14	464	26.3
D6	7.7	6.0	14	462	41.2

* The lipocaic (lot no. 47240) was furnished by Eli Lilly and Co.

TABLE 2

The effect of feeding lipocaic derived from 100 grams of raw pancreas on liver fat of completely depancreatized dogs maintained with insulin

DOG NO.	WEIGHT		LIPOCAIC			LIVER	
	Initial	Final	Lot no.*	Amount fed daily	Period fed	Weight	Total fatty acids
	kgm.	*kgm.*		*gm.*†	*wks.*	*gm.*	*per cent*
D7	10.2	10.4	H-7240	2.54	8	510	7.74
D8	10.2	9.7	H-7423	0.50	9	353	18.7
D9	13.0	9.6	H-7104A	0.37	15	411	3.87
D10	13.6	9.5	H-7104A	0.37	16	442	3.60
D11	14.1	10.5	H-7104A	0.37	16	925	26.9
D12	12.7	9.7	H-7423	0.50	16	722	47.5
D13	10.7	11.0	H-7104A	0.37	20	770	41.6
D14	8.2	4.7	H-7423	0.50	20	550	43.9
D15	11.9	7.8	H-7423	0.50	20	610	38.1

* Furnished by Eli Lilly and Co.

† The amounts recorded in this column were derived from 100 grams of raw pancreas.

dogs D13, D14 and D15, although they received daily for 20 weeks an amount of lipocaic equivalent to 100 grams of raw pancreas.

II. *Blood Lipids.* The fall in cholesterol, phospholipids and total fatty acids of the blood that occurs in completely depancreatized dogs kept alive by insulin and a diet containing no raw pancreas was observed in 1934–35 (13, 14). It was later shown that the addition of raw pancreas to the diet not only prevented

this fall but also led to a rapid and pronounced rise in all blood lipid constituents when it was fed to depancreatized dogs in which the blood lipids had fallen to low levels (15). Since the maintenance of a normal lipid content in the liver of the completely depancreatized dog by the daily feeding of raw pancreas was always associated with a blood lipid level that was either normal or above normal, the effects of lipocaic on the blood lipids were tested.

TABLE 3

Effect of lipocaic on blood lipids

DOG	PERIOD AFTER PANCREATECTOMY	WEIGHT	DAILY DIET*						SAMPLE ANALYZED	LIPIDS—MGM. PER 100 CC. OF PLASMA OR WHOLE BLOOD					
			Meat	Sucrose	Raw pancreas	Lipocaic‡		Period on diet weeks		Cholesterol			Total fatty acids	Phospholipids	Total lipids
						Pancreas equivalent				Total	Free	Ester			
	weeks	*kgm.*	*gm.*	*gm.*	*gm.*		*gm.*								
D16	Before (2)†	14.2	460	20	none	none		4	Plasma	229	77	152	507	281	736
	2	12.5	500	100	250	none		2	Plasma	212	76	136	515	370	727
	6	9.8	500	100	none	100	0.50	4	Plasma	147	80	67	379	220	526
D14	Before (0)†	8.2	240	20	none	none		2	Plasma	235	70	165	578	395	813
	4	6.8	500	100	250	none		4	Plasma	268	85	183	554	460	882
	12	6.1	500	100	none	100	0.50	8	Plasma	65	24	41	210	128	275
D15	Before (2)†	11.9	380	20	none	none		4	Plasma	247	78	169	618	403	865
	2	10.6	500	100	250	none		2	Plasma	293	86	207	689	407	982
	10	10.1	500	100	none	100	0.50	8	Plasma	145	62	83	390	225	535
D8	Before (0)†	11.2	360	20	none	none		4	Plasma	244	89	155	555	277	799
	3	9.6	500	100	250	none		3	Plasma	249	79	170	558	365	807
	11	8.7	500	100	none	100	0.50	8	Plasma	69	34	35	163	73	232
D9	Before (0)†	14.1	370	20	none	none		5	Whole blood	193	127	66	462	447	655
	2	13.3	500	100	250	none		2	Whole blood	220	133	87	473	482	693
	17	9.6	500	100	none	100	0.37	15	Whole blood	147	70	77	405	390	552
D12	Before (0)†	13.2	395	20	none	none		4	Plasma	231	79	152	574	360	805
	4	12.1	500	100	250	none		4	Plasma	272	83	189	677	420	949
	21	9.7	500	100	none	100	0.50	17	Plasma	160	51	109	517	252	677
D13	Before (0)†	10.6	300	10	none	none		3	Whole blood	177	122	55	402	437	579
	3	9.9	500	100	250	none		3	Whole blood	194	112	82	441	475	635
	23	11.0	500	100	none	100	0.37	20	Whole blood	101	88	13	264	268	365

* The amounts recorded were fed in 2 meals at 8:00 a.m. and at 4:00 p.m. Vitamin and salt supplements were added as described in text.

† Figures in parentheses show the interval before pancreatectomy: (0) refers to the morning when the pancreas was excised; (2) refers to 2 days before pancreas was removed.

‡ Lot no. of the lipocaic fed to D9 and D13 was H-7104A; the other dogs received lot no. H-7423. It was prepared by Eli Lilly and Co.

In table 3 are shown the results obtained in 7 dogs. The following lipid constituents were measured: cholesterol, both free and esterified, phospholipids and total fatty acids. For the first 2 to 4 weeks after pancreatectomy each dog received 250 grams of raw pancreas daily in addition to the lean meat, sucrose and supplements of salts and vitamins. The feedings of raw pancreas were then discontinued and the feeding of lipocaic substituted. The feeding of an amount

of lipocaic derived from 100 grams of original pancreas was continued for periods varying from 4 to 20 weeks.

Blood samples were taken for the determination of lipid constituents 1, on the day the pancreatectomy was performed or 2 days preceding this operation; 2, at the end of the feeding of raw pancreas, and 3, at the end of the next 4 to 20 weeks during which time lipocaic was fed.

An examination of the first 2 lipid determinations recorded for each dog in table 3 shows that the feeding of raw pancreas served to maintain lipids of all 7 dogs at normal levels for the first 2 to 5 weeks after pancreatectomy. Thus at the time the feeding of lipocaic was begun the blood concentration of cholesterol, both free and esterified, phospholipids, and of total fatty acids of all dogs was normal. The third blood determination, made at the end of a variable period during which lipocaic was fed, is therefore a test of the capacity of lipocaic to maintain normal lipid levels.

The earliest examination of the effects of lipocaic was made in dog D16. The blood lipids of this dog were determined 4 weeks after the daily feeding of lipocaic was begun. By this time a pronounced fall had occurred in total cholesterol, cholesterol esters, phospholipids and total fatty acids. The concentration of total lipids fell from 736 (preoperative value) to 526 mgm. per cent.

In three dogs (D14, D15 and D8) plasma lipids were determined after an 8-week period during which lipocaic was fed daily. The plasma lipid values found at the end of this 8-week period were considerably below their respective normal values in all 3 dogs. In dog D14 total cholesterol fell from a preoperative value of 235 mgm. to 65 mgm. and total fatty acids from 578 mgm. to 210 mgm. The drop in plasma lipids was equally striking in dog D8.

The effects of a still longer period of lipocaic feeding were tested in dogs D9, D12 and D13. These dogs were fed lipocaic for 15, 17 and 20 weeks respectively. In these, too, lipocaic failed to prevent the fall in blood lipids. Twenty weeks after lipocaic feeding was begun in dog D13, total fatty acids fell from 402 mgm. to 264 mgm., phospholipids from 437 mgm. to 268 mgm., and total lipids from 579 mgm. to 365 mgm.

The above results demonstrate that an amount of lipocaic prepared from 100 grams of pancreas does not maintain the blood lipids of depancreatized dogs at normal levels. One hundred grams of raw pancreas, however, contain more than enough of the blood lipid-raising factor to prevent the blood lipids from falling below preoperative or normal levels. Indeed, it was found in this laboratory that as small an amount as 5.5 grams of pancreas is sufficient to keep total fatty acids, phospholipids, and total lipids at normal levels even when fed as long as 20 weeks (16). Thus in 3 dogs that were tested in a similar manner to those recorded in table 3, the lipid values found before operation and 20 weeks after the daily feedings of 5.5 grams of pancreas were, respectively: total fatty acids, 402 and 462, 437 and 720, 405 and 523; phospholipids, 428 and 466, 475 and 588, 411 and 453; total lipids, 600 and 600, 631 and 984, 611 and 683. The blood lipids of these 3 dogs were examined at least 5 times during the 20-week interval in which the pancreas was fed; at no time were the total fatty acids,

phospholipids or total lipid content of the blood found below the preoperative levels.

COMMENTS. The results obtained here leave no doubt that lipocaic is a poor source of the anti-fatty liver factor of the pancreas. This conclusion is based on the failure of lipocaic to prevent the development of fatty livers in completely depancreatized dogs when this preparation is fed in amounts equivalent to 100 grams of original pancreas for periods as long as 20 weeks. Six of the 9 dogs that were fed this amount of lipocaic for 8 to 20 weeks had fatty livers. Three of these dogs were fed lipocaic for 20 weeks, at the end of which time their livers contained 38 to 42 per cent fatty acids. The amount of lipocaic fed was extracted from an amount of pancreas more than enough to prevent the development of fatty livers. As stated above, as small an amount as 5.5 grams of this glandular tissue is sufficient to maintain a normal lipid content in the liver of the completely depancreatized dog (3).

SUMMARY

1. Lipocaic (Dragstedt's preparation of pancreas) was tested for its ability to prevent the development of fatty livers in completely depancreatized dogs maintained with insulin. As judged by this test, lipocaic is a poor source of the anti-fatty liver factor of the pancreas.

2. The administration of lipocaic equivalent to 100 grams of pancreas to completely depancreatized dogs failed to prevent the fall of cholesterol, phospholipids or total fatty acids of the blood below preoperative levels.

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ORTHOSTATIC CIRCULATORY FAILURE ("GRAVITY SHOCK") IN THE DOG[1]

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Received for publication December 27, 1943

In a previous publication (1) we reported that although anesthetized dogs are usually able to withstand relatively long periods in the upright, feet down position, all compensation disappears when the carotid sinuses are denervated and the vagi cut. The frequency and ease with which irreversible failure or shock could be precipitated suggested that further investigation of orthostatic circulatory failure might contribute interesting information to the general problem of shock.

The general plan of the present experiments was similar to that previously described. The animals, anesthetized with chloralose (40–80 mgm. per K. B. W.) or with Na barbital (180–250 mgm. per K. B. W.), were rotated about a transverse horizontal axis, the position of which was adjusted in each case to coincide with the axis of the carotid cannula used in conjunction with a calibrated membrane manometer for recording blood pressure. The distance from the point of arterial cannulation to the fourth interspace (heart level in the upright position) was measured and the value thus obtained applied as a correction for the hydrostatic pressure effect to all blood pressure readings made with the animal in the upright position. Respiration was recorded either by a pneumograph or by a small T-tube inserted directly into the thoracic cavity and connected with a sensitive tambour. Wintrobe tubes were used to determine the hematocrit values of blood drawn from the femoral artery and heparinized. Specific gravity of whole blood and plasma was also determined in most of the experiments by the falling drop method. Protein content was calculated from these values, using the formula of Weech, Reeves and Goetsch (2). Gas analyses by the Van Slyke and Neill method were also carried out in six experiments on arterial blood drawn from the femoral or carotid arteries and from the femoral or jugular veins. The thoracic lymph duct was cannulated in six experiments and the flow of lymph and its specific gravity was measured. Lymph protein was determined gravimetrically in several experiments and the values were found to check closely with those calculated from specific gravity determinations. During the operation for cannulation of the thoracic duct, prior to the collection of lymph, physiological saline (20 cc. per K. B. W.) was given intravenously to insure adequate hydration of the tissues.

The usual procedure was to make adequate control observations with the animal in the horizontal position, after which the animal was tilted to an angle of

[1] Aided by a grant from the John and Mary R. Markle Foundation.

75°, feet down, and kept in this position for varying periods of time during which the observations were continued. The tilting usually took from 1 to 2 seconds. In some experiments the animals were returned to the horizontal position and the observations continued. However, many of the animals succumbed in the upright position or failed to recover when returned to the horizontal.

Representative results are shown in figures 1 and 2. They suggest that the changes which occur in orthostatic circulatory insufficiency or "gravity shock" are similar, in many respects, to those reported as taking place in traumatic shock (3). Dogs, allowed to remain in the upright, feet down (F. D.) position for 20 minutes to 4 hours, show an eventual drop in blood pressure to levels of 30 to 60 mm. Hg. This hypotension is accompanied by definite and often marked increases in the hematocrit and blood specific gravity values. In some cases, the hemoconcentration begins within a few minutes after the animal is

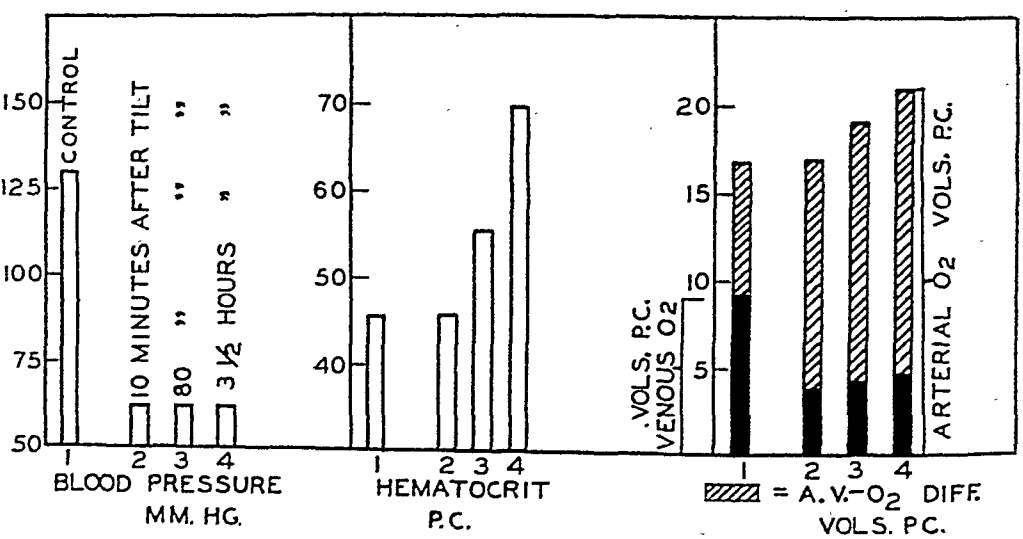

Fig. 1. Experiment 2. Dog weighing 10 K anesthetized with 250 mgm. Na barbital K. B. W. Died 3 minutes after drawing of last blood sample.

tilted (fig. 2); in others, there is no significant change until 20 to 30 minutes later (fig. 1). The hemoconcentration usually increases gradually as long as the F. D. period is maintained and shows a slow gradual fall toward the original pre-tilt level when the animal is returned to the horizontal position.

The oxygen content of arterial blood reflects the increased hemoconcentration and no anoxemia is evident even in samples taken just before death in experiments lasting several hours or more (fig. 1). The venous blood, however, shows a sharp drop of 50 to 100 per cent within 10 to 15 minutes after the animal is tilted. This results in a rise of 40 to 100 per cent in the A-V oxygen difference. This evidence of a retardation of blood flow through the tissues confirms the results of our previous experiments in which the blood flow in the larger arteries and veins was measured by means of thermostromuhrs (4). It does not, however, support our suggestion (5) that the erect position causes an anoxemia which stimulates the chemoreceptors.

Along with the increase in the A-V oxygen difference, there also occurs a parallel and quantitatively similar increase in the A-V carbon dioxide difference. This is due to a marked (22 to 56 per cent) drop in femoral or carotid arterial blood content accompanied by a smaller decrease (18 to 40 per cent) in the CO_2 content of blood drawn either from the femoral or jugular veins. These changes are not necessarily associated with an increased CO_2 elimination, as shown by the results

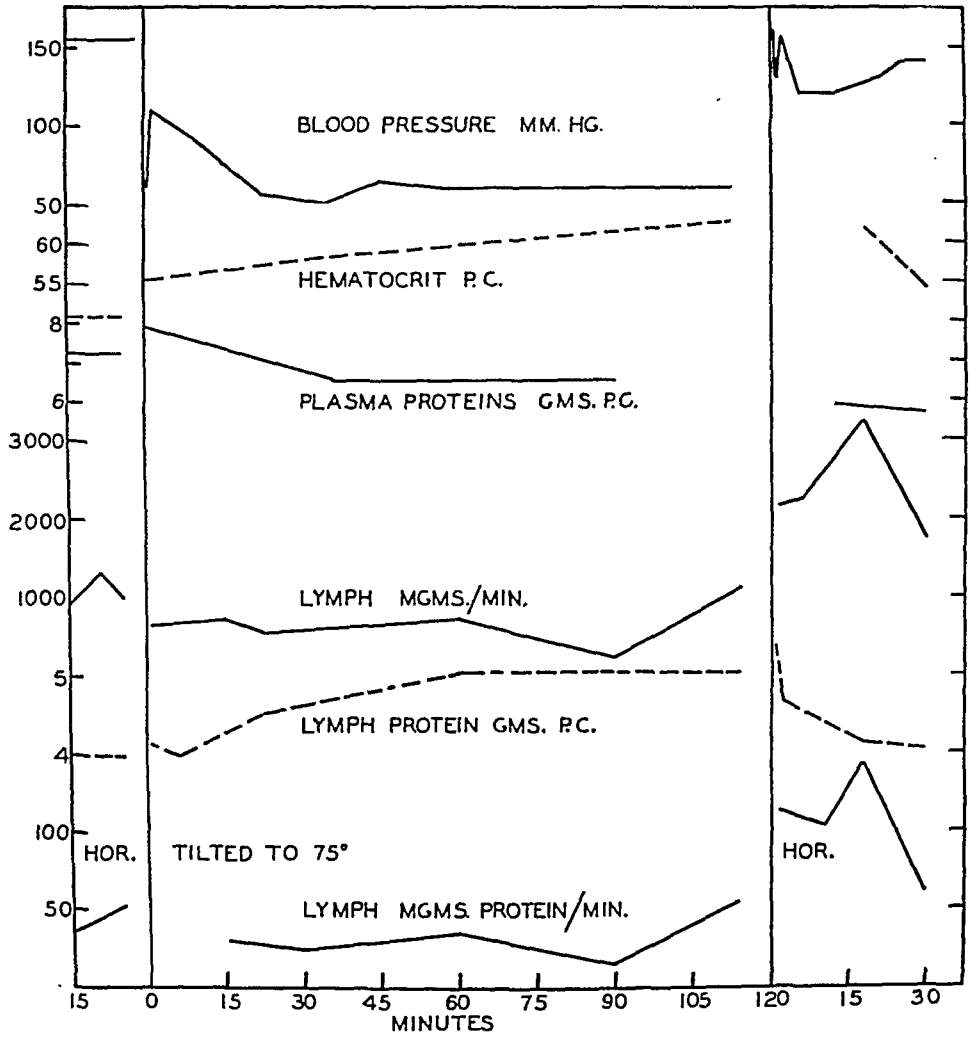

Fig. 2. Experiment 8. Dog weighing 15 K anesthetized with 250 mgm. Na barbita K. B. W. 300 cc. 0.9 per cent NaCl infused 3 hours before experiment started.

of two experiments in which the pulmonary ventilation and CO_2 content of the expired air were measured. Similar results have been described in experiments on man and their explanation remains obscure (6, 7). Mackay (8) has recently proposed that the main factor responsible for the fall in alveolar CO_2 is a reduction in the volume of blood to which the lung gases are exposed.

While the increases in the hematocrit and plasma protein values are similar for the first five to ten minutes after tilting, this parallelism disappears as the F. D. period is prolonged. Analysis of the data of 18 experiments shows that, as the

hemoconcentration proceeded, the plasma protein level rose slightly in 3 cases, remained at the same level in 5 cases and actually diminished appreciably in 10 experiments. The six experiments in which lymph protein was measured also provide evidence that as the F. D. position is maintained, the continued sluggish flow and consequent hypoxia result in a greater or less increase in capillary permeability and consequent loss of protein from the blood stream (fig. 2). Although the rate of lymph flow is reduced during the F. D. position (or absent when the blood pressure level is 40 mm. Hg or lower), its protein concentration is increased. Return of the animal to the horizontal position and recovery of the blood pressure level always results in a striking increase in the flow of lymph, which usually reaches a peak in about 15 minutes. At this time the rate of flow and the actual amount of protein in the lymph are at least two to three times that found previous to tilting. Readjustment toward pre-tilting levels takes place in about a half hour. Similar changes, indicative of a temporary increase in capillary permeability, were also found by Maurer (9) after a period of low oxygen ventilation and by McCarrell and Drinker (10) after histamine injections. In order to investigate the possibility that the changes might be due to the liberation of histamine, samples of blood and lymph from two experiments were assayed for histamine by Dr. Gerhard Katz of the Department of Pharmacology. He was unable to find any significant change in the histamine content of blood or lymph in any of the samples obtained during the F. D. period.

Originally, it was planned to tilt the animals and study the blood changes while they were suspended in the F. D. position for relatively long periods of time and then to precipitate shock, if it had not already developed, by eliminating the moderator reflexes. The extreme sensitivity of the animals to even slight hemorrhage, however, made this impossible. The removal of the small amounts of blood necessary for the successive determinations almost invariably precipitated shock. Thus in experiment 2 (fig. 1) a total of 20 cc. of blood had been withdrawn during the 3½ hours of the F. D. period. At this time an additional 10 cc. was withdrawn for sampling. The blood pressure, which had remained at 60 mm. Hg throughout the period, now fell rapidly and, although the animal was quickly returned to the horizontal position and artificial respiration and infusion of saline begun, the animal died within 3 minutes of respiratory failure.

The following typical experiment strikingly illustrates the critical level of circulating blood volume which is achieved and maintained in animals kept in the F. D. position for long periods of time. A 6.5 K. dog (expt. 15) was tilted and kept in the F. D. position for 30 minutes. The blood pressure dropped from 170 mm. Hg to 100 mm. Hg, rose quickly to 140 mm. and then slowly diminished to 70 mm. at the end of the half-hour F. D. period. On return of the animal to the horizontal position, the blood pressure rose and remained at a level of about 115 mm. Hg for the next 90 minutes. The animal was then tilted again. The blood pressure dropped to between 30–50 mm. Hg. Fourteen minutes after tilting, 10 cc. of blood was removed from the carotid artery. The respiration stopped almost immediately and the dog showed a typical asphyxial rise in blood pressure. Rapid infusion of heparinized blood obtained from a second animal was

started as soon as the blood pressure began to fall. The level of blood pressure rose immediately and remained at 110 mm. after the infusion of 150 cc. of blood. Respiration, however, did not begin again until seven minutes later. The withdrawal of 10 cc. of blood fifteen minutes after the completion of the infusion resulted in a drop in blood pressure to only 90 mm. Hg. On return of the animal to the horizontal position, the pressure rose to 140 mm. Hg. This level was maintained and did not change subsequent to the removal of 10 cc. of blood. The dog was then tilted for the third time. The usual immediate drop in blood pressure did not occur. The pressure remained at 140 mm., rose to 150 mm., dropped gradually to 110 mm. at the end of 30 minutes. Withdrawal of 10 cc. of blood at this time resulted in a drop in blood pressure of only 5 mm.

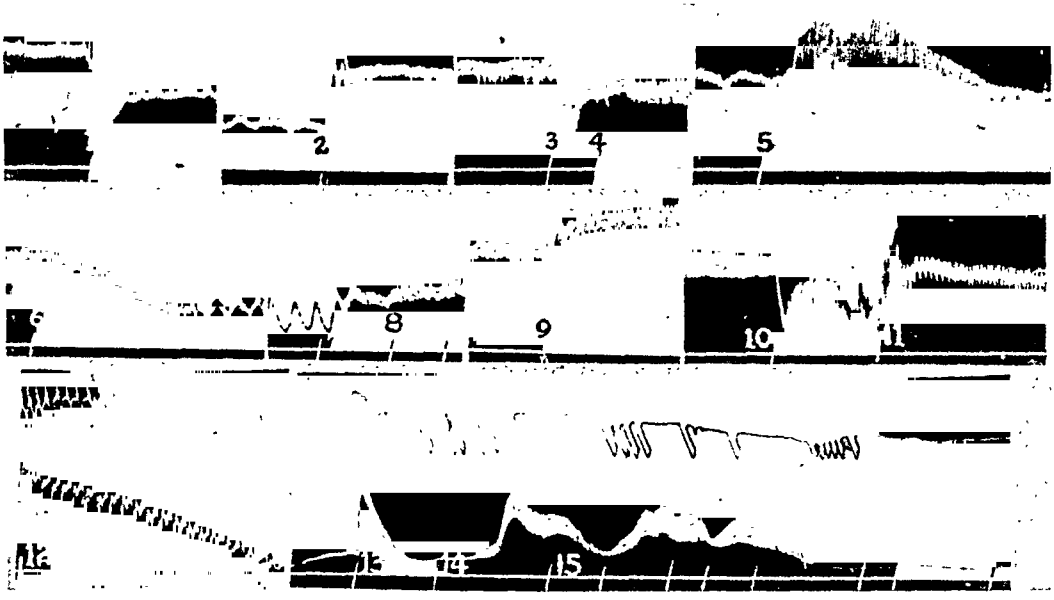

Fig. 3. Experiment 27. See text for details. Top line of lowest strip is respiration record.

Further indication of the importance of the blood volume was obtained in the six experiments in which lymph flow was studied. As previously mentioned, these animals were primed with 300 to 500 cc. of physiological saline before the experiment so as to insure adequate hydration. These dogs showed a much better response to tilting and were not sensitive to slight hemorrhages, particularly in the early part of the F. D. period.

Additional experiments also showed that if infusions are maintained during the F. D. period, the usual blood pressure drop can be entirely eliminated. One of these experiments is shown in figure 3. The dog, weighing 6 K and anesthetised with 250 mgm. Na barbital per K. B. W. was tilted at 1. The blood pressure fell from 130 mm. Hg to 65, increased to 90 and then diminished again to 40 mm. at the end of 10 minutes, when the animal was returned to the horizontal—2. The dog was then infused with a total of 400 cc. of physiological saline given over a period of 30 minutes and again tilted at 3. At 4, the infusion was resumed and continued at a constant rate of 20 cc. per minute for 60 minutes. The initial fall

in blood pressure was similar to that observed for the first tilt, but instead of a rise and secondary fall, the pressure now rose continuously. At the end of 10 minutes, it was 100 mm. Hg and after 30 minutes was at 110 mm. Hg. At 5, adrenalin (5 cc. of a 1 to 100,000 solution) was added to the perfusion fluid and produced a typical response. The pressure returned and stayed at the same level (110 mm. Hg) for seventy minutes after the infusion had been stopped. At this time —6, 100 cc. of blood was removed, resulting in a drop in blood pressure to 45 mm. Hg. At 7, infusion of saline was begun and at 8, the 100 cc. blood (which had been heparinized) was reintroduced. The blood pressure rose and was at 125 mm. Hg (slightly below pre-tilt level) when the animal was returned to the horizontal at 9. The dog had been maintained in the F. D. position for 3 hours and 54 minutes.

The blood pressure obtaining at this time was high, 175 mm. Hg, reflecting the increase in blood volume. It dropped gradually, due presumably to the diffusion of the saline to the tissues. At the end of 30 minutes, the pressure was at 130 mm. Hg, the original level at the beginning of the experiment. The animal was then again tilted—10—and showed the usual fall in pressure. At 11, the vagi were severed and at 12, the left carotid clipped to eliminate temporarily the action of the carotid sinus. The pressure fell rapidly to 20 mm. Hg and the respiration became periodic. The animal was then returned to the horizontal position—13—(duration F. D. period = 16 min.), and the clip removed from the carotid. The pressure rose to 100 mm. Hg but fell rapidly again. At 14, infusion of saline was begun and at 15, blood was introduced and its infusion continued without, however, preventing the death of the animal several minutes later. Variations of this procedure in other experiments, on the other hand, showed that irreversible failure does not occur when the blood volume is increased by the infusion of saline or blood prior to or concomitant with the elimination of the moderator nerves, or when the infusion is maintained throughout the F. D. period.

In his classic reports on the effects of posture, Hill (11) maintained that the fall in blood pressure in the dog was primarily due to blood accumulating in the splanchnic area and that the compensation which occurred was due to splanchnic vasoconstriction. Edholm (12), however, showed that, in the cat, evisceration diminished the vascular compensation but did not abolish the fall and that removal of the liver almost completely abolished the fall of blood pressure in the F. D. position. Edholm concluded that the reason for the fall of blood pressure is not the collection of blood in the splanchnic area, but in the liver, and that the splanchnic area is only partly responsible for the compensation following this fall. Repetition of some of his experiments suggests that a similar situation exists in the dog, i.e., that the decrease in circulating blood volume is chiefly due to pooling of blood in the liver. The recovery of the blood pressure when the animal is restored to the horizontal is due, in large measure, to the return to the right side of the heart of the accumulated blood in the liver. The effect of gravity may also be enhanced by a constriction of the hepatic veins such as is presumed to occur in anaphylactic or peptone shock in the dog. The action of adrenalin, as seen in our experiments, may therefore be partly the result of a relaxation of these veins

following a constriction of the liver vascular bed and an immediate delivery into the general circulation of a considerable quantity of blood (11). It should be emphasized in this connection that, while pooling in the liver is of greater importance than pooling in the legs in the cat and dog, the situation is probably the reverse in man because of the different relative proportions of the lower limbs.

Our results and those of other investigators (6) demonstrate that orthostatic circulatory failure or "gravity shock" is primarily initiated by a decrease in the effective circulatory blood volume. This is the result of increased filtration on the arterial (capillary) side and stagnation and pooling on the venous side. The consequent drop in blood pressure evokes a reflex compensatory vasoconstriction via the moderator nerves which opposes further flooding of the capillary reservoirs. This serves to diminish the capacity of the capillary system and makes more blood avilable for the general circulation. The attendant slowing of the blood flow, however, tends to increase capillary stasis and plasma loss when the F. D. position is maintained for more than short periods of time. A delicate balance is often achieved whereby the blood pressure is maintained, even for hours, at a low level, but remains adequate to prevent lasting damage to tissues. Irreversible failure is precipitated as a result of any change which upsets this new balance by further diminishing the circulating blood volume. Elimination of the moderator reflexes results not only in a diminished peripheral resistance but also in an enlarged capacity of the vascular bed, thus further reducing the effective circulating blood volume below the already critical level. Hemorrhage in the F. D. position can no longer evoke its usual compensatory vasoconstriction, since the vessels have already been contracted as a compensation to the position. Withdrawal of small amounts of blood, therefore, easily reduces the circulating blood volume to below the adequate level.

SUMMARY

Anesthetized dogs, suspended in the upright (feet down) position for 20 minutes to 4 hours, show varying degrees of hypotension, progressive hemoconcentration and marked increases in arterio-venous oxygen and carbon dioxide differences. After 10 to 20 minutes, the plasma protein level diminishes while the protein concentration of lymph increases. These changes can be reversed by returning the animals to the horizontal position and approximately control values are achieved in about thirty minutes.

Animals kept in the upright position are extremely sensitive to hemorrhage. Irreversible failure is often precipitated by the withdrawal of relatively small quantities of blood (30–50 cc.). This can usually be prevented and the response to tilting improved by the infusion of saline and/or blood during the upright period.

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THE PATTERNS OF THE ARTERIAL PRESSURE PULSE

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Received for publication January 8, 1944

During the past ten years there have accumulated in this laboratory a large number of records of the arterial pressure pulses taken with the Hypodermic Manometer (4). These are faithful records of the changes in the pressure of the blood in various arteries of man and of other animals. The form of the pressure pulse curve is quite variable. Some of the types are drawn roughly to scale in the figures presented herewith, but there are many intergrading examples which can not be shown for lack of space.

It is the purpose of this paper to describe the manner in which these typical patterns take form and to present a simple hypothesis that will account physically for the variations seen in the pressure pulses in different arteries and under different conditions.

The simplest pulse form is that seen in very small animals such as the mouse, canary, frog or turtle (1). During early systole the ejection from the heart is rapid, blood is coming into the arterial tree faster than it is leaving through the arterioles and the pressure in the arteries rises abruptly. Later the rate of ejection falls, the arterial pressure rise continues but becomes less abrupt. Toward the end of systole there is a balance between the rate of ejection and the rate of arteriolar drainage. The curve is then horizontal. An actual decline in the curve is sometimes seen during late systole, when ejection falls to a rate that is less than arteriolar drainage.

During diastole the curve is a perfectly smooth fall of pressure with time. It falls faster at first when the pressure is high. Blood drains out of the arterioles more rapidly and each unit of blood leaving the arterioles makes a greater pressure difference when the pressure is high than when the pressure is low.

This smooth pressure curve is the simple consequence of filling and emptying of the arterial compression chamber. However it is seen only in small animals. The tiny arteries of these creatures respond so quickly that they follow faithfully the pressure changes which are produced by the heart.

In ordinary laboratory animals such as the dog and in man the form of the pressure pulse is much more complex. Superimposed upon the first described filling and emptying curve are waves of arterial origin. This is because the mass of the arterial blood column is so large and the arterial walls are so distensible that the action of the heart sets up oscillations of pressure in the artery that are reflected back and forth over its length as they gradually damp out. The natural period of these waves varies directly with the time it takes the pulse wave to be transmitted from one end of the artery to the other and their amplitude depends upon the abruptness of the initial upstroke of the pulse and the sharpness with which reflection takes place at the end of the artery. These waves, therefore,

differ in different arteries and in different parts of the same artery. They differ with the degree of peripheral constriction, with changes in the blood pressure and can be modified by occluding (pinching) the artery beyond the place where the manometer needle is inserted.

The most important of these waves of arterial origin is the great standing wave of the aorta (2). This wave oscillates about a node which is situated in the descending thoracic aorta. When the wave is reaching its trough above this node it is reaching its peak below. It is called a standing wave because the wave reaches its peaks and troughs simultaneously in all parts below the node. Similarly, above the node the peaks and troughs are simultaneous all the way up the ascending, transverse and descending aorta and even in the ventricle during systole. The wave is called a free pressure oscillation because it is the dying down of a highly damped surging which is determined by the elastic characteristics of the arterial tree and the mass of blood within. The damping factor is supplied by the viscosity of the blood and by incomplete reflection.

Since it is difficult to record the pressure pulses from the node and thus make a recording of the fundamental filling and emptying curve of the aortic compression chamber, it is convenient in many experiments to derive this curve from records taken from points above and below the node. In figure 1 this has been done using curves from the root of the brachiocephalic artery of the dog and from the femoral. The brachiocephalic curve was made with a sound down the carotid artery connected with a hypodermic manometer and the femoral curve was made from a needle inserted into the artery so as not to occlude flow and connected to another manometer. The two curves were redrawn to the same pressure and time scales.

It is seen that the two curves interlace very much as would the curves of two tuning forks, one vibrating strongly and one vibrating weakly and 180° out of phase. The base line on which the oscillations are inscribed goes up and down with the arterial filling and emptying. A smooth curve can then be drawn through the points of intersection to approximate the fundamental filling and emptying curve of the arterial compression chamber. Theoretically it should be closer to the brachiocephalic than to the femoral curve because the latter has the stronger oscillations. It should have the same area as both and should look something like the dotted curve in figure 1. The damped oscillation drawn in below is in phase with the difference between the femoral and fundamental filling and emptying curve.

The standing waves of the carotid artery are superimposed not upon the fundamental filling and emptying curve of the arterial tree but rather upon the pressure curve which actually occurs in the root of the aorta. The standing wave of the aorta and that of the carotid are therefore superimposed upon the fundamental filling and emptying curve to make the pulse pattern as it is seen in the carotid artery.

The carotid standing waves themselves are seen in figure 2. They begin to appear 2 or 3 cm. from the arch and are simultaneous, peak and trough, from there up to the head. They are, therefore, standing waves of the same sort as

those in the aorta. The amplitude of the carotid standing waves may be increased by vasoconstriction and also by pinching the artery between the fingers peripheral to the manometer needle. Their frequency can be increased somewhat by raising the blood pressure (which increases the rate of pulse wave transmission) or greatly and progressively by pinching the artery closer and closer to the needle which is in the root of the neck. This shortens the resonating column over which the pulse wave must be transmitted. It will be noticed from figure 2 that the free vibrations are set off not only by systole itself but also by the aortic pressure changes at the incisura.

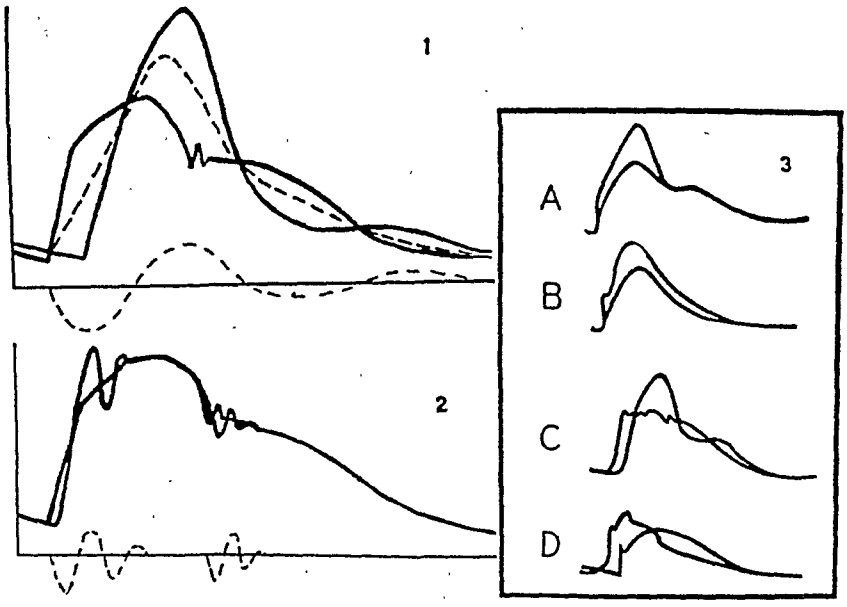

Fig. 1. Comparison of the femoral (peaked curve) and aortic pressure pulses. An approximation of the fundamental filling curve of the aortic tree is dotted in. Below is a rough estimate of the free pressure oscillations of the aortic tree.

Fig. 2. Comparison of the pressure pulses in the root of the aorta, and of the carotid artery which starts later and has the transient pulses. Below is a rough estimate of the free pressure oscillations of the carotid tree.

Fig. 3A. Pressure pulses from the two femorals a few seconds after one (the lower curve) has been injected with a small dose of acetylcholine.

B. The same ten seconds later.

C. Pressure pulses in carotid and femoral.

D. The same after a small intravenous dose of acetylcholine.

The relation of the pulse pattern in the aorta and the fore leg arteries has not been determined in the dog. In man the arm arteries do not usually give clear evidence of a separate standing wave system unless the artery has been occluded below the needle (3). Very occasionally there is a brachial standing wave which is recognizable. Its frequency is greater than that of the aorta but considerably less than that of the carotid.

In the dog the hind leg arteries follow the standing wave system of the aorta, the peak and dips being simultaneous all the way down from the aortic node to the dorsalis pedis. In man the dorsalis pedis and femoral pulsations are not

simultaneous (4). They seem to differ according to the same principles as do the aortic and carotid curves. The free pressure oscillations of the large leg arteries are of course much slower than those of the carotid.

Occluding the artery below the manometer needle produces a sharp point of reflection and sets up standing waves of different form than the natural waves. The fact that during occlusion the systolic pressure usually reaches greater height than normal has given rise to the fiction of "systolic end pressure." The rise in pressure (when it occurs) central to the point of occlusion is due to three factors. First, there is an increase in the peripheral resistance over the whole body because the occlusion shunts the blood which would ordinarily go out the arterioles of the stopped artery into the remaining arterioles and it takes a few millimeters more of pressure to force the cardiac output through the fewer arterioles. This is an increase of both systolic and diastolic pressure and is not usually maintained very long. It is regulated against reflexly. Secondly, there is the moot question as to whether the velocity head may not appear as pressure when the artery is occluded. If the artery in question is a branch of the aortic tree, occluding the artery makes it a part of the manometer which measures the pressure below the bifurcation and and in the larger trunk where a certain part of the energy is in the form of velocity and a certain part in the form of pressure. Conditions are so complex that it seems impossible to say whether the balance between these two energies will be significantly changed by occluding the branch. Certainly the amount of velocity energy which had been at the needle point is irrelevant in explaining the change in the pressure pulsation upon occlusion. The third and principal factor in explaining the increase in systolic pressure upon occlusion of the artery is the result of setting up an entirely new set of standing waves. These are conditioned by the fact that the artery is stopped and have no relation to pressures or energies present in the unstopped artery.

Normally, the waves of the oscillating aortic system are reflected at one end from the heart or from the semi-lunar valves. At the other end they are reflected, not from large bifurcations but from the terminal arterioles. In these small vessels there is classically an increased resistance to flow which is the physical condition for the reflection of pulsatile waves (2). In case of the secondary systems such as the carotid and human leg arteries the site of peripheral reflection is the same as that of the aortic system, i.e., the arterioles. The central site of reflection is probably the opening of the artery into the aorta which acts as the open end of a sounding tube. In the sense that fluctuations of pressure are minimal and fluctuations of movement maximal it is analogous to a node.

To show that it is the peripheral resistance which is responsible for the reflected waves it is necessary to produce a vasodilation in the peripheral bed of the artery under examination. If this can be done without changing the level of blood pressure and without producing any great change in the manner of wave reflection in other arteries the experiment will be more convincing.

The two femoral arteries were exposed and the needles of two hypodermic manometers (4) inserted in each so as not to obstruct flow of blood. The pulse curves were identical and showed a systolic peak that was 30 or 40 mm. higher

than the systolic pressure in a simultaneous carotid pressure pulse (fig. 3 C). Now a very small dose of acetylcholine was injected into the right femoral artery. The systolic peak in this artery disappeared at once and the pressure during systole became 30 or 40 mm. lower in the right femoral artery than in the left (fig. 3 A). During diastole the pressure in the two femoral arteries was equal, showing that the arteriolar run off was not sufficiently great in the dilated bed to make a continued difference in pressure between the two femorals. The difference seemed to be due simply to the absence of the systolic peak in the injected artery. The systolic pressure pulse in the left femoral artery was almost unaffected and the second wave (negative) was reduced somewhat but equally in both arteries. A few seconds later the second wave had disappeared from both arteries (fig. 3 B). The wave reflected from the periphery had been lessened by general vasodilatation so that the second wave cannot be seen. The drug had worked no change in the arterial wall which prevented the transmission of reflected waves because these waves reappeared when the leg was grasped tightly at the knee making a pseudo-vasoconstriction. They could also be brought back by intra-arterial injections of epinephrine in small doses which produced constriction of the dilated arterioles in the femoral bed.

When somewhat larger doses of acetylcholine are given intravenously the standing waves are eliminated from all arteries. The femoral pressure pulse curve is very similar in systolic height to that of the aortic arch, being delayed and smoothed out. In the carotid artery the initial standing waves are eliminated by dilatation in its peripheral bed but there appears another wave that is transmitted up the carotid artery appearing later in the upper parts of the vessel. In appearance it is not very different from the carotid standing wave but it is not always a continuation of the upstroke of the carotid pulse nor is it repetitive as are the standing waves (fig. 3 D). It only occurs when the diastolic pressure in the arterial system as a whole is very low and the arterial column becomes more or less stationary between heart beats. When ejection begins against the inertia of this stationary column, there is a sudden rise of pressure in the aorta and its lower branches. At the same time there is a surge of pressure which travels out the carotid and which accounts for the wave in question.

The sudden increase of pressure in the lower arteries gives rise to the pistol shot sound which can be heard over the artery whenever diastolic pressure gets so low that flow is definitely reduced in rate in these vessels. (Aortic regurgitation, A.V. fistula, etc.) The carotid surge seems to be a sort of back fire from the water hammer that is responsible for the pistol shot sound.

The presence or absence of reflected waves can be used in hypotension to determine the cause of the fall in blood pressure. If it is due to reduced circulatory blood volume and occurs in spite of vasoconstriction, the reflected waves can clearly be recognized. If the primary cause of the low blood pressure is vasodilatation or if a terminal vasodilatation occurs its indication can be seen from the absence of reflected waves from the pressure pulse curves.

As the blood pressure falls during an experiment the pulse wave transmission time increases and the frequency of the standing waves decreases. The heart

may accelerate and the second wave be partly or wholly obliterated from the femoral pressure pulse. The second wave of the carotid (or preferably upper aortic) pulse then is the only easy guide to the presence of reflected waves because the femoral systolic pressure may be a little higher than that at the root of the aorta. The reciprocal relationship between such curves is shown in figure 4 and is an indication that there is enough peripheral resistance to cause the reflection of the waves.

On the other hand, blood pressure may be low because of generalized relaxation of arterioles and the reflected waves thereby be eliminated. The arteriolar relaxation may be due to direct action of drugs, to stimulation of depressor receptors from certain internal injuries such as exploration in the upper abdominal region. The relaxation may also result from vasoconstrictor "exhaustion" that follows long continued low blood pressure. In these cases the typical relationship of the pressure pulses is shown in figure 5. The femoral and aortic contours are then very similar and there are none of the reciprocal relationships indicating

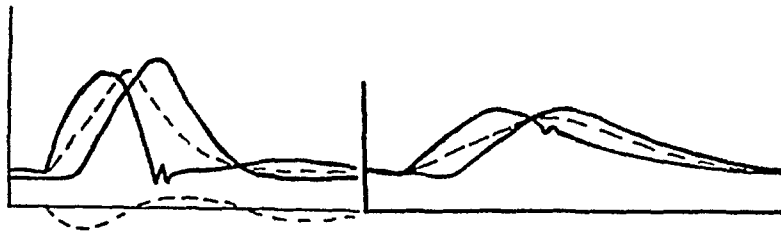

Fig. 4 Fig. 5

Fig. 4. Curves from the aorta and femoral artery showing reflected waves. The dog has a low blood pressure (65/40).

Fig. 5. Curves from the aorta and femoral artery showing absence of reflected waves. The dog has a low blood pressure (60/45).

standing waves which are seen when the blood pressure is low in spite of vasoconstriction. The carotid surge and quick upstroke which are responsible for the pistol shot sound (see fig. 3 D) may not be present if the circulation has seriously deteriorated.

Studies of pulse contours offer certain advantages over plethysmography in determining the degree of vasodilatation and hence in helping to decide whether low pressure is due to vascular relaxation or to oligemia accompanied by vasoconstriction. When blood pressure is extremely low the pulsation of and the blood flow into the various peripheral organs is low whether there is peripheral constriction or not. This is the direct consequence of the greatly reduced blood flow (cardiac output) and this in turn results from the low venous pressure. The venous pressure may be low whether the blood volume is reduced or whether the vascular bed is relaxed.

SUMMARY

The pressure pulse patterns in various arteries are described in terms of the filling and emptying of the arterial tree and the added reflected waves which are

contributed by the various arteries. It is shown that these waves are reflected from constricted arterioles and that the form of the pulse may be used to evaluate the rôle of vasodilation in producing hypotension.

This investigation was helped by a grant from the American Medical Association.

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CARDIAC INSUFFICIENCY IN THE VITAMIN E DEFICIENT RABBIT[1]

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Received for publication December 10, 1943

Since 1928, when Evans and Burr (1) first reported the occurrence of muscular paralysis in rats deprived of vitamin E, their findings have been duplicated in almost every conveniently available species of laboratory animal. The literature of this period has been thoroughly covered by Pappenheimer in his recent review (2).

In young rabbits, placed on a diet virtually free of vitamin E at about 5 weeks of age, the development of the dystrophy follows a very characteristic course. At two to three weeks the animals begin to exhibit muscular weakness which increases with extreme rapidity to paralysis and death in 24 to 48 hours, many of them dying in the pre-paralytic stage. The life expectancy of any of these animals can be estimated with a high degree of accuracy at any time within this period by observation of the extent of spontaneous activity, the tone of the body musculature and their resistance to handling, their ability to right themselves when displaced from the normal posture, etc.

Since man or experimental animals can live for long periods with much more extensive paralysis than that observed in most of our animals at the time of death, and since the lesions in the skeletal muscles are generally admitted to be the most serious of any which have been described thus far in vitamin E deficiency (2), we became interested in determining, if possible, the actual immediate cause of death.

If it is true, as most comparative studies seem to indicate, that "the more fundamental chemical energetics of striated and cardiac muscle are identical" (3) it did not seem reasonable to us that a nutritional dystrophy could proceed to the point of paralysis of the skeletal muscles without simultaneous involvement of the heart. Moreover, the suddenness of death lends support to the view that it is the result of acute circulatory failure. We have therefore investigated this possibility by the use of two procedures, one capable of precipitating failure in a weakened heart, the other designed to support a heart in which myocardial failure is imminent.

EXPERIMENTAL. New Zealand White rabbits, at 5 weeks of age, weighing about 750 grams, were separated into groups by dividing litter-mate pairs. One group was placed on a vitamin E deficient diet (4), while the second group received either their normal ration or the deficient diet plus alpha-tocopherol acetate. When muscular dystrophy became severe the animals received either

[1] Supported by a grant from the funds made available by the Legislature for Research in the University of Oklahoma School of Medicine, Oklahoma City.

posterior pituitary extract[2], or one of the cardiac glycosides[3], intravenously by way of the marginal vein of the ear. A dystrophic animal and its litter-mate control were given the same drug as nearly simultaneously as possible, and the duration of the survival period observed.

In table 1 we have recorded the effects of posterior pituitary on 7 vitamin E deficient and 7 normal animals. A dose of 0.1 cc. was immediately fatal to six

TABLE 1

The effect of posterior pituitary on normal and vitamin E deficient animals

	Animal		Dose		Survival following administration of drug	
	Number	Weight	Individual	Total	Immediate death	No effect
Vitamin E deficient						
		gm.	*cc.*	*cc.*	*minutes*	
Posterior pituitary	D-1	960	0.1	0.1	5½	
	D-2	1030	0.1	0.1	5	
	D-3	700	0.1	0.1	6	
	D-4	730	0.1	0.1	1	
	D-5*	720	0.1, 0.2, 0.2	0.5		Death at 3 hours—not significantly different from expectancy if if untreated. See note
Pitressin	D-18	900	0.1	0.1	5	
	D-19	850	0.1	0.1	4	
Normal controls						
Posterior pituitary	N-1†	1560	0.2, 0.1(5), 0.2 0.2, 0.5	1.6		Complete recovery
	N-2†	1630	0.3, 0.2, 0.4	0.9		Complete recovery
	N-3	1030	0.3	0.3	5	
	N-4†‡	730	0.2, 0.2, 0.3, 0.4	1.1		Complete recovery
	N-5‡	840	0.3	0.3		Complete recovery
Pitressin	N-17	1100	0.2	0.2		Complete recovery
	N-18	1100	0.3	0.3		Complete recovery

* Doses at 10 minute intervals. Severe reaction after each dose.
† Successive doses at 5 minute intervals. Little reaction to any except the first.
‡ Received vitamin E deficient diet plus adequate dl alpha-tocopherol acetate.

of the dystrophic animals. The seventh died three hours after having received a total of 0.5 cc., in three doses at 10 minute intervals. In contrast, 6 of the 7 normal animals survived an initial dose two or three times as large. Three of

[2] Posterior Pituitary Solution Squibb (surgical strength); Pituitary Extract, Surgical, Sharp and Dohme; or Pitressin, Parke, Davis & Co.

[3] Ouabain (G-strophanthin) Merck, or Digoxin (glycoside of digilanid C from Digitalis Lanata), Burroughs, Wellcome and Co.

them received additional doses at 5 minute intervals, one receiving a total of 0.9 cc. within a 10 minute period. Successive doses of equal size usually evoke progressively diminishing responses in the normal animal, so we attach no importance to the number given; but the final dose was large enough in each of these cases to emphasize the normally high resistance to the drug.

The dystrophic animals reacted to the initial dose with a brief period of struggling, very much limited in intensity by their muscular weakness. This was followed by complete collapse; the heart beat could not be detected through

TABLE 2

The effect of digoxin on normal and vitamin E deficient animals

Dose—0.5 mgm. (1 cc.) per kgm. See notes

ANIMAL		PREDICTED SURVIVAL	SURVIVAL FOLLOWING ADMINISTRATION OF DRUG		
Number	Weight		Immediate death	Extended survival	
			Vitamin E deficient		
	gms.				
D-6*†	1300	2 to 6 hours		3½ days	
D-7†	1000	1 to 2 hours		2½ days	
D-8	1000	1 to 2 hours		36 hours	
D-9	900	1 to 2 hours	5 min.		Collapsed during injection
D-10‡	1000	2 to 6 hours		24 hours	
D-11†	1100	2 to 6 hours		7 days	
			Normal controls		
N-6§	2000		2 hours		
N-7	1600		3½ hours		
N-8	1800		45 min.		
N-9	1600		45 min.		
N-10	1300		30 min.		
N-11	1000		15 min.		

* Initial dose of 0.25 mgm. per kgm., followed by 0.5 mgm. per kgm. 18 hours later.
† Paralysis complete after 24 hours; could not eat during remainder of period.
‡ Pulmonary edema at time of death.
§ Dose = 0.425 mgm. per kgm.

the chest wall, and the ears were completely blanched. After one or two minutes the heart beat again became palpable, its force gradually increasing, but with gross irregularities suggesting extrasystoles. With returning consciousness there was a brief outburst of convulsive activity, during which the heart stopped abruptly. Respiration usually persisted for a few cycles.

Normal animal N-3 struggled with extreme violence during and after the administration of the drug, and did not recover from the collapse which followed. With this exception none of the control animals suffered complete collapse; neither did the heart beat at any time become so weak as to be imperceptible.

In table 2 the protective action of digoxin on the heart of the vitamin E

deficient animal is contrasted with its lethal effect in normal controls. With the exceptions noted, these animals received the calculated minimum lethal dose; 0.5 mgm. per kgm., or 1.0 cc. per kgm., of the preparation used. On the basis of past experience we could predict death within 6 hours at the most for even the least affected of these E deficient animals. We believe, therefore, that this table is self explanatory and that the protective action of digoxin is amply demonstrated.

In table 3 a similar protective action by ouabain is demonstrated. Although in this case also the calculated minimum lethal dose was given (0.2 mgm. per kgm.), it did not prove to be the L. D. 100 for the normal control animals. The E deficient animals in this group were in an earlier stage of dystrophy, so

TABLE 3

The effect of ouabain on normal and vitamin E deficient animals

	ANIMAL NUMBER	SURVIVAL
Vitamin E deficient—wt. 750 to 1100 grams		
Ouabain, 0.2 mgm. per kgm.	D-12	Survived despite severe dystrophy and were killed 4 days after receiving drug
	D-13	
	D-14	Dead at 24 hours
	D-15	Died 2½ days after receiving drug
Controls—no drug administered	D-16	Dead 16 hours after time of administration of drug to other animals
	D-17	
Normal—wt. 850 to 1200 grams		
Ouabain, 0.2 mgm. per kgm.	N-12	Recovered completely
	N-13	
	N-14	Dead at 16 hours
	N-15	
	N-16	

the drug was withheld from two of them to obtain more accurate information as to their expectancy if untreated. The drug was administered in late afternoon. All the animals were living at 9 p.m. that same evening, but the two dystrophic controls and three of the injected normal controls were dead the next morning, 16 hours after the time of injection; some of the animals had been dead for several hours. Although two of the normal controls which received the drug recovered completely, it is obvious that all of the dystrophic animals which received it outlived their expectancy (less than 16 hrs.—see above) by hours or days.

Thoracic x-ray films were made on a number of these animals, both normal and dystrophic, in an effort to determine whether there were any significant

differences in heart size. The normals were deprived of food for 24 hours, after which dystrophic and normal animals of equal weight were chosen for comparison. Exposures were made at the standard tube distance of 30 inches. Visual comparison and rough measurements, after allowance for rotation and any other detectable artifacts, indicated some cardiac enlargement in the dystrophic animals. Our series is small, however, and since our present facilities are inadequate for a thorough investigation, we do not wish to emphasize this point.

An early attempt was made to obtain graphic records of the effect of these drugs on heart action but was abandoned because we had no method available at that time which did not require the use of an anesthetic.

Discussion. The lesions which occur in the voluntary muscles of vitamin E deficient rats were first described by Olcott (5), who reported that no pathology could be found in the heart. Morgulis, Wilder, Spencer and Eppstein (6) found no change in the lipid content of the heart in dystrophic rabbits although an increase in fat content is one of the most characteristic changes in the skeletal muscles. There are observations in the literature, however, which indicate that functional and metabolic changes may become apparent before pathological changes appear. Knowlton and Hines (7) found that the capacity for work could be reduced by as much as 50 per cent in the gastrocnemius muscles of rats on a vitamin E deficient diet at a time when only a few of the fibres showed hyaline changes. Numerous workers have demonstrated an increase in the resting oxygen consumption of dystrophic muscle from vitamin E deficient animals (4, 8, 9, 10), amounting to 200 to 400 per cent in some cases. Recently, Kaunitz and Pappenheimer (11) have shown that this increase becomes sizable in the muscles of vitamin E deficient young rats before any pathological changes are detectable, and one of us (O. B. H.) in unpublished experiments, has found that the oxygen consumption of heart muscle slices from E deficient hamsters may be increased as much as 40 per cent.

Two recent observations in the literature suggest an explanation for the ability of the heart to resist functional deterioration until the dystrophy is well advanced in the skeletal muscles. Mason (12) has shown that heart and lung tissue from normal animals may contain twice as much vitamin E as body fat, in which fat soluble substances might be expected to concentrate, or liver, which is notable as a storage reservoir for other vitamins. That the heart may be able to retain its more adequate supply in the face of depletion elsewhere is indicated by the observations of Hines and Mattill (13), who found that tocopherol added to minced tissue could not be recovered quantitatively without the addition of sulfuric acid.

Eventually, however, as our experiments show, the heart is reduced to the failing state through a cycle of events which we visualize as follows: Depletion of vitamin E leads to an increased oxygen demand and a reduced capacity for work. This increased oxygen requirement is obviously independent of heart volume, since it has been found to occur in isolated skeletal muscle and heart slices. The reduced capacity for work, however, leads to dilatation, which superimposes a further increase in oxygen requirement.

Thus the vitamin E deficient, failing heart becomes exceedingly sensitive to any reduction in its oxygen supply. Posterior pituitary extract, through the action of pitressin, greatly reduces the coronary blood flow (14) and precipitates the crisis which we have described.

It is generally accepted that the failing heart, particularly if it is dilated, is more resistant to the toxic effects of the cardiac glycosides than is the normal (15, 16, 17), and that a dose of digitalis which may be beneficial to such a failing heart may have dangerous or even fatal consequences in normal individuals due to a reduction of cardiac output or to disturbances of the normal cardiac rhythm. Since the majority of our vitamin E deficient animals lived for several days beyond the predicted time of death after receiving digoxin or ouabain, they were obviously benefited. The actions of digitalis to which this benefit may be ascribed are first, direct stimulation of the myocardium, with an increased cardiac output and a reduction of heart volume resulting from the greater strength of systole; second, an increase in efficiency which results in a greater work output at a given rate of oxygen utilization (18).

Since it is recognized that cardiac function may be impaired in such deficiency syndromes as beri-beri and pellagra, we are tempted to suggest that vitamin E, which has come to be recognized as a specific factor in the maintenance of normal metabolism in striated muscle, may also play a rôle of major importance in the maintenance of normal cardiac function in man.

SUMMARY

We have shown that rabbits in a state of nutritional muscular dystrophy as the result of vitamin E deprivation exhibit the following signs of severe myocardial damage:

1. A greatly increased sensitivity to posterior pituitary extracts; they are killed by doses much smaller than those which are well tolerated by normal control animals.
2. A high resistance to the toxic effects of the cardiac glycosides; their lives are preserved for several days beyond the predicted time of death in the majority of cases, by doses of digoxin or ouabain which are lethal to normals.
3. Probable cardiac dilatation, as revealed by thoracic x-ray films.

From these findings we conclude that the sudden death of vitamin E deficient animals in an advanced stage of muscular dystrophy is due directly to myocardial failure.

The authors wish to acknowledge their indebtedness to Dr. Ernest Lachman of the Department of Anatomy, who made the x-ray films from which the information on heart size was obtained.

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THE EFFECTS OF HEXOSES ON THE RESPIRATORY EXCHANGE OF RHESUS MONKEYS[1]

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Received for publication December 20, 1943

The present investigation is a part of the Nutrition Laboratory's general program of research on the biological variations in the response of the respiratory exchange to ingestion of glucose, levulose, and galactose. Because of the similarity of the monkey to man in several aspects of its physiology, the study of the effect of ingestion of hexoses on the respiratory exchange of the monkey is a logical part of the general program.

METHODS AND PROCEDURE. The respiratory exchange[3] was determined by the open-circuit chamber principle, which involved measuring the total ventilation, aliquot sampling of the outgoing air, and analysis by means of an accurate gas analysis apparatus (Carpenter, 1933). The accuracy of the analyses was controlled by analyses of outdoor air or air from burning ethyl alcohol. Seven adult female rhesus monkeys were used. They were deprived of food during 24 hours before each experiment, and they had been for at least 24 hours at an environmental temperature of 22° to 29° and were measured at a chamber temperature of about 28°C. Water and sugar solutions, at 39°C., were given by stomach tube. Five series of experiments were carried out, namely, with no dose and minimum amount of handling, after administration of 105 cc. of water given in portions of 70 and 35 cc. immediately following one another, and after administration of 14 grams of glucose, fructose, or galactose in 70 cc. of water plus 35 cc. for rinsing funnel and tubing. The amount of sugar given was calculated, for comparative purposes, on the basis that the dose for the monkey should have the same ratio to a dose of 100 grams for an adult 70-kgm. man as the basal oxygen consumptions of these two (about 35 and 250 cc. per minute, respectively). The measurements were begun within 18 to 27 minutes after the dose and continued for 8 consecutive periods of 30 minutes each. In the comparisons made in the following pages between the effects of hexoses on the metabolism of the monkey and that of man, the statements regarding man are based on articles of Carpenter and his co-workers, which are cited in the bibliography at the end of this article.

RESULTS. *Respiratory quotient (R.Q.).* In the 3 experiments with no administration of sugar and minimum amount of handling, the R.Q.'s in the

[1] A preliminary report was presented at the fiftieth annual meeting of the American Physiological Society in Baltimore, Md., in 1938; This Journal **123**: P32, 1938.

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[3] The measurements of the respiratory exchange and the gas analyses were made at the Baltimore laboratory by Mr. Basil James, formerly of the Nutrition Laboratory staff.

first two periods were, except in one instance (no. 265, first period), lower than in any of the periods following (table 1). In spite of care in handling the monkeys, they were probably excited during the introduction into the chamber with the result that during the first hour there was an overventilation, which was

TABLE 1

Respiratory quotients of rhesus monkeys after ingestion of hexoses

KIND OF EXPT. AND DATE (1937)	MONKEY NO.	RESPIRATORY QUOTIENT (MINUTES AFTER DOSE)							
		0–30	30–60	60–90	90–120	120–150	150–180	180–210	210–240
No dose, no handling									
Nov. 24	265	0.73	0.68	0.73	0.72	0.74	0.76	0.75	0.75
Nov. 29	393	0.65	0.71	0.75	0.76	0.75	0.76	0.75	0.76
Nov. 15	430	0.71	0.71	0.73	0.75	0.75	0.75	0.76	0.76
Water, 105 cc.									
Nov. 1	73	0.73	0.72	0.77	0.76	0.76	0.77	0.77	0.77
Nov. 22	97	0.82	0.83	0.84	0.84	0.85	0.85	0.84	0.83
Nov. 26	97	0.66	0.72	0.72	0.72	0.73	0.72	0.73	0.72
Oct. 22	245	0.64	0.70	0.75	0.75	0.77	0.75	0.75	0.74
Nov. 8	434	0.66	0.71	0.75	0.72	0.75	0.74	0.80	0.74
Glucose, 14 gm.									
Oct. 25	73	0.91	0.98	1.00	1.04	1.01	1.00	1.01	0.98
Oct. 28	97	0.82	0.95	1.00	1.00	1.01	0.92	0.83	0.76
Nov. 5	245	0.71	0.81	0.87	0.91	0.89	0.90	0.87	0.83
Nov. 9	265	0.78	0.93	0.95	0.98	0.95	0.91	0.88	0.89
Nov. 23	430	0.88	1.01	1.02	0.99	0.92	0.86	0.82	0.79
Fructose, 14 gm.									
Oct. 29	245	0.86	0.96	0.95	0.94	0.88	0.80	0.79	0.79
Nov. 19	265	0.73	0.77	0.80	0.78	0.79	0.76	0.74	0.73
Nov. 3	393	0.72	0.81	0.88	0.87	0.75	0.77	0.78	0.77
Nov. 10	430	0.76	0.87	0.82	0.77	0.77	0.77	0.76	0.76
Oct. 26	434	0.77	0.90	0.91	0.91	0.90	0.90	0.78	0.77
Galactose, 14 gm.									
Nov. 4	97	0.70	0.74	0.77	0.78	0.79	0.79	0.80	0.77
Nov. 16	97	0.85	0.84	0.82	0.81	0.80	0.80	0.80	0.79
Nov. 11	245	0.73	0.81	0.84	0.85	0.85	0.84	0.86	0.84
Nov. 12	265	0.73	0.79	0.82	0.79	0.81	0.80	0.81	0.78
Oct. 27	393	0.66	0.76	0.78	0.78	0.75	0.77	0.76	0.76
Nov. 2	434	0.82	0.90	0.90	0.91	0.90	0.91	0.90	0.90

followed by a compensatory lowering of the R.Q. From the third half hour on, the R.Q. varied between 0.72 and 0.76, and for each experiment the level was reasonably constant. With 105 cc. of water without sugar the results are somewhat similar to those with no dose, with the exception of the quotients for no. 97 on November 22. This animal may have obtained food in some unknown way.

After the ingestion of 14 grams of glucose there was an immediate rise in R.Q. in 4 of 5 experiments, which masked the depressing effect of handling shown in the series with no dose and with water. In the second half hour the rise was marked in all 5 experiments, and the R.Q. continued at a high level in all experiments through the sixth half hour and in two of them throughout the 4 hours. The results indicate that glucose was metabolized rapidly and that there was a marked increase in carbohydrate combustion or even possibly some conversion of sugar to fat. Glucose caused a greater rise in the R.Q. of the monkey than in that of man. The peak for the monkey came at the third to fifth half hour, which is about the time it would occur with man after 40 to 100 grams of glucose. The fall after the peak was slightly more rapid with the monkey than with man.

The ingestion of 14 grams of fructose was accompanied by an immediate rise of the R.Q. in the first half hour in 3 of 5 experiments, and the maximum rise occurred in all of them by the third half hour. The rise was not so large and sustained as with glucose, and the fall was more rapid than with glucose. The results with the monkey are different from those with man, as fructose produces in man a greater rise in R.Q. than does glucose, with a resulting greater apparent combustion of sugar. The peak with monkeys was in the second and third half hours, slightly later than with man.

There was more uniformity in the rises in the monkeys' quotients after glucose or fructose than after galactose. In the latter case the R.Q.'s showed little or no rise above the base line in 3 experiments, marked rises in 2 experiments, and an abrupt rise followed immediately by a decrease in one experiment. This lack of uniformity among the monkeys in their response to galactose is similar to the finding with women, who showed more variability than men in the R.Q. after ingestion of galactose. On the average, the maximum rises in the R.Q.'s of the monkeys were smaller after galactose than after the other two sugars and smaller than the marked rises found with both men and women after 40 or 50 grams of galactose. However, the maximum rises in the monkeys' quotients were sustained for a longer time after galactose than after glucose or fructose. Moreover, the maximum quotients of the monkeys persisted for 3 to 3½ hours after galactose, whereas with man the maximum was reached in about 1 hour and thereafter the quotients fell rapidly. As 3 monkeys from which it was possible to obtain urine samples showed excretion of sugar in the urine after galactose ingestion, it is probable that monkeys, like humans, do not have a high tolerance for galactose.

Oxygen absorption. The values of the oxygen absorption for the successive half-hour periods are given in table 2. In general, the highest values are found in the first half hour, probably as the result of previous activity and excitement. The values fall thereafter slightly but continuously or reach a constant level in the remaining half hours. The results are presented to enable the calculation of the heat production, and detailed discussion is unnecessary.

Proportion of heat production supplied by carbohydrates. The total heat production during the 4 hours of measurement has been calculated from the R.Q. and the total oxygen absorption. The amount of carbohydrate burned and the

proportion of the total heat production supplied by carbohydrate have been estimated on the assumption that the oxygen required for protein combustion was 15 per cent of the average basal oxygen consumption in the experiments with no dose. On this basis it was calculated that the average urinary nitrogen

TABLE 2

Oxygen consumption of rhesus monkeys after ingestion of hexoses

(Liters per 30 minutes)

KIND OF EXPT. AND DATE (1937)	MONKEY NO.	OXYGEN CONSUMPTION (MINUTES AFTER DOSE)							
		0–30	30–60	60–90	90–120	120–150	150–180	180–210	210–240
No dose, no handling									
Nov. 24	265	1.61	1.22	1.24	1.19	1.15	1.13	1.12	1.11
Nov. 29	393	1.33	1.00	1.09	0.98	1.03	1.01	1.03	1.00
Nov. 15	430	1.73	1.15	1.11	1.23	1.17	1.14	1.10	1.11
Water, 105 cc.									
Nov. 1	73	1.20	1.11	1.18	1.23	1.09	1.15	1.13	1.13
Nov. 22	97	1.72	1.45	1.36	1.33	1.31	1.29	1.30	1.28
Nov. 26	97	1.43	1.07	1.07	1.03	1.06	1.08	1.04	1.02
Oct. 22	245	1.50	0.99	0.91	0.97	0.91	0.89	0.89	0.89
Nov. 8	434	0.99	0.85	0.97	0.97	0.85	0.83	1.02	0.83
Glucose, 14 gm.									
Oct. 25	73	1.64	1.32	1.24	1.31	1.29	1.29	1.33	1.37
Oct. 28	97	1.28	1.11	1.08	1.08	0.99	0.96	1.00	1.04
Nov. 5	245	1.13	0.90	0.86	0.82	0.85	0.83	0.81	0.86
Nov. 9	265	1.42	1.15	1.14	1.08	1.20	1.10	1.13	1.10
Nov. 23	430	1.85	1.38	1.34	1.20	1.20	1.20	1.17	1.22
Fructose, 14 gm.									
Oct. 29	245	1.11	0.98	0.97	0.98	0.90	0.84	0.86	0.83
Nov. 19	265	1.57	1.23	1.19	1.14	1.18	1.03	1.07	1.10
Nov. 3	393	1.50	1.22	1.19	1.12	1.09	1.04	1.03	1.01
Nov. 10	430	1.81	1.43	1.25	1.28	1.25	1.17	1.18	1.16
Oct. 26	434	1.30	1.11	1.06	1.01	1.04	1.04	0.92	0.91
Galactose, 14 gm.									
Nov. 4	97	1.49	1.19	1.18	1.15	1.16	1.08	1.00	1.04
Nov. 16	97	1.61	1.22	1.28	1.19	1.22	1.22	1.19	1.17
Nov. 11	245	1.17	0.88	0.87	0.91	0.83	0.85	0.83	0.86
Nov. 12	265	1.40	1.27	1.14	1.20	1.18	1.14	1.09	1.13
Oct. 27	393	1.47	1.16	1.19	1.14	1.15	1.16	1.13	1.13
Nov. 2	434	1.18	0.91	0.90	0.96	0.93	0.94	0.98	0.92

elimination of these monkeys was 0.31 gram per kilogram of body weight per 24 hours. This value is supported by similar values calculated from urinary nitrogen data for monkeys reported in the literature (Hunter and Givens, 1914; Friedemann, 1934) and was used in all the calculations of carbohydrate combustion. The results are given in table 3. In the two series with no dose and with

water the energy supplied by carbohydrate was under 15 per cent, with the exception of no. 97 on November 22. After the ingestion of glucose the values range from 42 to 89 per cent, after fructose from 14 to 53 per cent, and after

TABLE 3

Heat production related to carbohydrate combustion of rhesus monkeys

KIND OF EXPT. AND DATE (1937)	MONKEY NO.	BODY WEIGHT	PER 4 HOURS		
			Total heat production	Carbohydrate combustion	Proportion of heat from carbohydrate combustion
			calories	*grams*	*per cent*
No dose, no handling					
Nov. 24	265	4.7	46.1	0.5	4
Nov. 29	393	4.5	39.9	0.3	3
Nov. 15	430	4.6	46.0	0.7	6
Water, 105 cc.					
Nov. 1	73	4.0	43.7	1.3	12
Nov. 22	97	5.2	53.5	5.1	40
Nov. 26	97	4.6	41.3	0.0	0
Oct. 22	245	3.5	37.4	0.2	2
Nov. 8	434	3.9	34.5	0.3	4
Glucose, 14 gm.					
Oct. 25	73	4.1	54.3	11.4	89
Oct. 28	97	4.7	42.2	6.4	64
Nov. 5	245	3.6	34.2	3.4	42
Nov. 9	265	5.0	45.9	6.7	62
Nov. 23	430	5.0	52.2	8.1	66
Fructose, 14 gm.					
Oct. 29	245	3.6	36.6	4.6	53
Nov. 19	265	4.8	45.3	1.5	14
Nov. 3	393	4.5	44.1	2.5	24
Nov. 10	430	4.9	50.4	2.7	23
Oct. 26	434	4.2	40.9	4.4	46
Galactose, 14 gm.					
Nov. 4	97	4.5	44.1	1.5	14
Nov. 16	97	4.9	48.6	3.8	33
Nov. 11	245	3.6	34.8	2.9	35
Nov. 12	265	4.8	45.7	2.6	24
Oct. 27	393	4.7	45.1	1.1	10
Nov. 2	434	4.2	37.9	5.2	58

galactose from 10 to 58 per cent. The ingestion of sugar in all three instances resulted in a greater proportion of heat derived from carbohydrate than was the case when no sugar was given. With man with no dose or with water, carbohydrate supplied from 27 to 43 per cent of the energy output in $2\frac{3}{4}$ to $3\frac{1}{2}$ hours, 50 grams of glucose supplied, on the average, 53 per cent and 104 grams 62 per

cent in $2\frac{3}{4}$ to 4 hours, 50 grams of fructose 62 per cent and 104 grams 78 per cent in $2\frac{3}{4}$ to $3\frac{1}{2}$ hours, and 50 grams of galactose 56 per cent in 3 hours. With man the order with respect to proportion of heat production supplied by carbohydrates was fructose, galactose, and glucose. With the monkeys glucose gave the highest proportion, whereas fructose and galactose gave somewhat smaller values but about equal to each other.

Increases in heat production resulting from ingestion of hexoses and specific dynamic action. The percentage increases in heat production during the 4 hours after ingestion of the sugars have been calculated, not from the average results for each series but by comparison of the heat production of the individual monkey after each of the sugars with the heat production of the same monkey in the no dose or the water experiment. With all but 2 monkeys the results show, on the average, increases in heat production of from 12 to 13 per cent in the three series with sugars. The specific dynamic action of the sugars (the percentage relationship between the fuel value of the sugar and the increase in heat production above the base line during the 4 hours of measurement) is estimated to average 11.3 per cent for glucose, 9.5 per cent for fructose, and 9.0 per cent for galactose. The specific dynamic action of these sugars (50 to 100 grams) with man was, on the average, somewhat lower.

SUMMARY

The respiratory exchange of 7 adult female rhesus monkeys was determined for 4 hours after no dose, after 105 cc. of water, and after the administration by stomach tube of 14 grams of glucose, fructose, or galactose. Glucose caused the greatest rise in R.Q., fructose the next greatest, and galactose the least. The order with man is fructose, galactose, and glucose. With the monkeys the proportion of heat production supplied by carbohydrates was the greatest after glucose; the proportions were somewhat smaller after fructose and galactose but about equal to each other. With man the order in this respect is fructose, galactose, and glucose. All three sugars caused increases in the heat production of 5 of the 7 monkeys of 12 to 13 per cent over that in experiments with no dose or water. The specific dynamic action of these sugars was about 9 to 11 per cent, which is somewhat more than that with humans.

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THE EFFECT OF ALUMINUM HYDROXIDE GEL ON GASTRIC SECRETION

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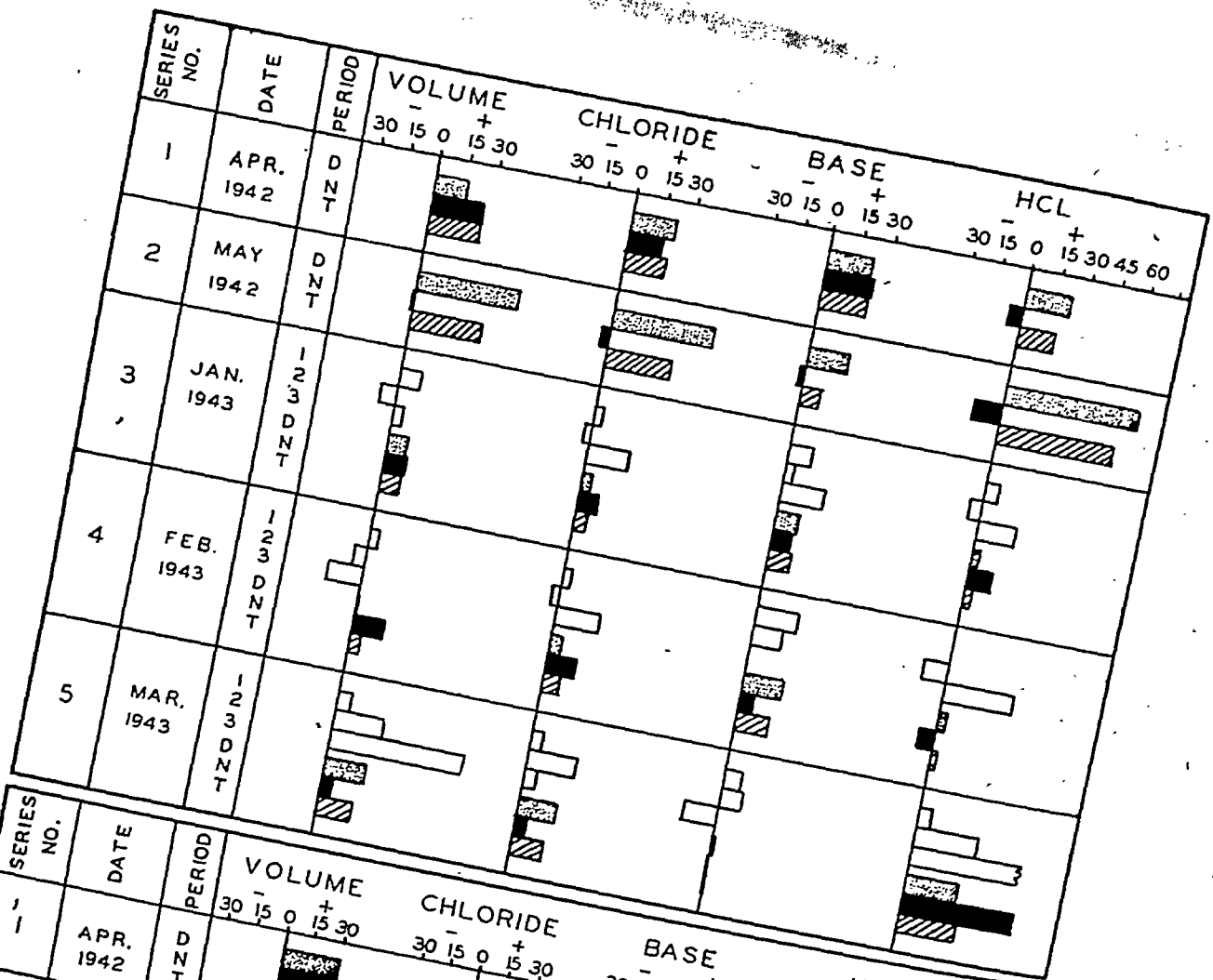
Received for publication December 22, 1943

The observation that an increase in acid *concentration* frequently occurs during fractional gastric analysis after the administration of certain antacids has led to the concept of "rebound" or "secondary" acid secretion. However, the effect of antacids on the *amounts* of secretion and of free hydrochloric acid has remained largely obscure. Our investigation of this aspect of the problem with $NaHCO_3$ has been reported (1) and we are now presenting a similar study of the effect of aluminum hydroxide gel on gastric secretion.

The important position of aluminum hydroxide gel among antacids rests on a secure foundation of experimental and clinical reports. It is generally agreed that this antacid has no rebound effect, and the recent report of Komarov and Kreuger (2) indicates that it may decrease secretion. Working with a Pavlov pouch dog which also had a fistula from the exterior into the stomach, they found that very large doses of aluminum hydroxide gel (60–90 cc.) diminished the secretory response. Doses up to 40 cc. administered just after a test meal were without effect, but when 20 cc. were administered 15 minutes before the test meal and a second 20 cc. were given $1\frac{3}{4}$ hours after the meal, the volume of secretion was markedly decreased without effect on acidity. Since their results were obtained chiefly with massive doses of aluminum hydroxide gel on individual days of experimentation, we have investigated the effects of this antacid in moderate dosage when it was administered three times daily after a test meal for five consecutive days.

EXPERIMENTAL. The routine and methods previously reported, using the Cope pouch dog and a technic developed in this laboratory (1), were employed in this study. Wartime conditions, however, required a change in dog food after the second series of experiments. Canned Bovex was replaced by a dehydrated product sold under the same name, but of different composition (originally a fish base). The new food was mixed each morning with the usual supplements of NaCl and HCl and sufficient water was added to approximate the consistency obtained originally with the canned food. Complete studies were carried out with the new food to determine that mixing the food in the morning, rather than at 4:00 p.m. on the previous day, produced no change in the secretory response.

Two Cope pouch dogs were used. Five series of experiments were made on each animal during a period of one year. Each series consisted of a week of control observations followed by a week of observations during which each animal received aluminum hydroxide gel by stomach tube at 9 and 11 a.m. and 2 p.m.



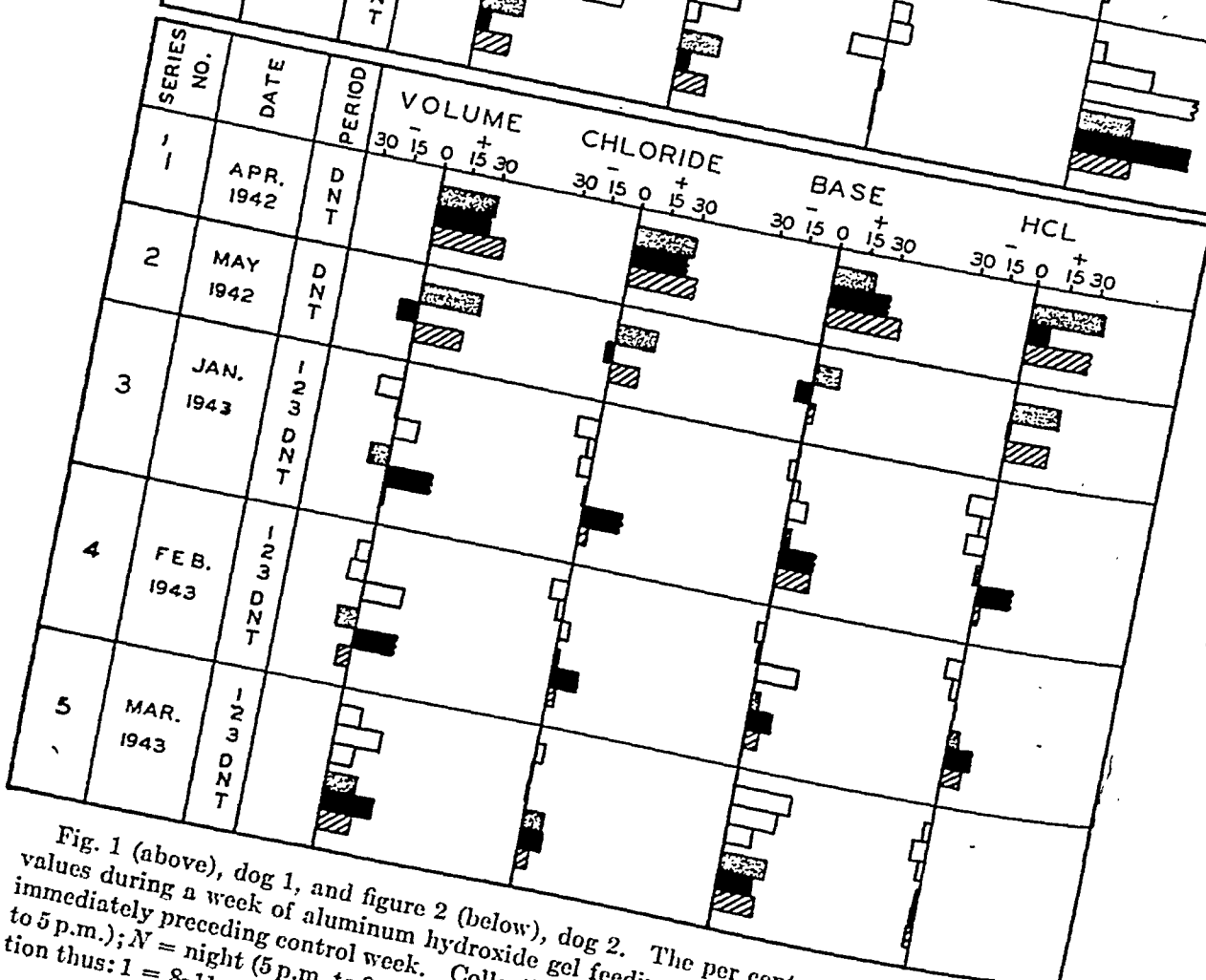


Fig. 1 (above), dog 1, and figure 2 (below), dog 2. The per cent of change of average values during a week of aluminum hydroxide gel feeding from the average values of the immediately preceding control week. Collection periods are indicated as D = day (8 a.m. to 5 p.m.); N = night (5 p.m. to 8 a.m.); T = total 24 hr., and as parts of the D (day) collection thus: 1 = 8–11 a.m., 2 = 11 a.m.–2 p.m., 3 = 2–5 p.m.

each day from Monday through Friday inclusively. Fifty cubic centimeters of a diluted gel, made by mixing 1 part of aluminum hydroxide gel (Amphojel) with 3 parts of water by volume, were given each time. A week of control observations also followed each week of gel feeding.

The pouch dogs were in excellent condition. Dog 1, a female, was 10 to 21 months post-operative and ranged from 10.3 to 11.2 kgm. in weight. This animal had a large pouch and on control routine secreted about 300 cc. of juice in 24 hours of which 50 to 75 cc. were secreted at night (5 p.m. to 8 a.m.). Dog 2, a male, was 9 to 20 months post-operative and weighed from 14.9 to 15.2 kgm. This animal secreted about 150 cc. in 24 hours but only 2 to 10 cc. at night. Because of the small night secretion in this dog small changes in night values appear as very large changes in per cent, and for this reason they are indicated in figure 2 by serrated edges to indicate the direction of change rather than the amount of change.

Results. The averages of data from three consecutive days during the week of aluminum hydroxide gel feeding were compared with similar averages of the preceding control week. These results are shown in figures 1 and 2 expressed in terms of the percentile deviation of averages of an experimental week from the corresponding averages of the preceding control week. In both animals during the first two series of study there were significant increases in the volume, total chloride and free acid, with less clear cut increases in total base. In the remaining three series neither animal showed significant changes from control, except dog 1, series 5, in which the free acid appears markedly increased. No consistent shift in secretory activity to an earlier period of the day is evident in these experiments as was seen with $NaHCO_3$ (1).

The results (not shown) of the control weeks following aluminum hydroxide gel administration are in close agreement with the corresponding control weeks which preceded the antacid administration.

The observations on hematology and blood chemistry showed no variation of significance.

Discussion. The explanation of the greater changes observed in series 1 and 2 in comparison with those of series 3, 4 and 5 is not apparent The only known variable between these groups of experiments was the change in the test meal. Perhaps the effect of certain antacids on gastric secretion is modified by the nature of the food used to stimulate secretion. Unfortunately the effects observed on the original food could not be reinvestigated. However, the administration of $NaHCO_3$ during the feeding of the new food has been repeated and our original findings were confirmed. This suggests that some factor entered into series 1 and 2 which was absent in the later series, and we place more confidence in the later observations. The increase in free acid observed in dog 1, series 5, cannot be exclusively attributed to the effect of the antacid, since this animal was isolated at the beginning of the week of antacid administration because of the appearance of oestrus. The observation that the administration of aluminum hydroxide gel produced no apparent effect on acid-base equilibrium agrees with numerous reports (3, 4, 5 etc.).

Under the conditions of these experiments, no depression of secretory activity was produced either during the administration of the aluminum hydroxide gel or in the control periods following antacid administration. These findings, therefore, are not in agreement with the observations of Komarov and Krueger (2).

In general these experiments indicate that aluminum hydroxide gel in moderate dosage produces no significant effect upon the amount of gastric secretion.

SUMMARY

The effect on pouch secretion of five consecutive days of feeding moderate doses of aluminum hydroxide gel three times daily after a test meal was studied using the Cope pouch dog.

No depression of secretory activity occurred either during or after the administration of the antacid.

The administration of moderate doses of aluminum hydroxide gel was accompanied by occasional increases in volume, total chloride, total base, and free acid of the pouch secretion, but in the majority of experiments these increases were too small to be significant.

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THE EFFECT OF VITAMINS AND SEX HORMONES ON DIETARY ACHROMOTRICHIA IN MICE

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Received for publication January 8, 1944

Recent investigations have shown that the graying of hair can be produced experimentally by such different factors as x-rays (1), the administration of certain chemicals (2) and also by vitamin B deficiency (Bakke et al. (3)). The latter authors believe that the loss of color of the fur results from an unknown substance present in the wheat germ rather than to a deficiency. Since then, investigators have focused attention on the influence of vitamins of the B complex on achromotrichia, especially p-aminobenzoic acid (4), pantothenic acid (5) and more recently folic acid (6), either alone or combined with other vitamins. The results are contradictory, due perhaps to species or strain difference of animals employed. Rats of weaning age were used in some investigations; they are more dependent on diet for rapid growth and may differ in this way from adult animals.

As reported earlier (7), C-57 male and female mice on a deficient diet respond differently. Fifty-five per cent of the males showed varying degrees of graying on the 30th day in contrast to 6 per cent of the females.

METHODS. Adult C-57 mice were caged in groups of 6 to 8. This strain in contrast to others can be raised and will reproduce on a synthetic diet (8). The diet supplied in these experiments was like that employed by Unna (9). Water was at all times accessible, reinforced with vitamins under test for prevention or cure of achromotrichia. Sex hormones in sterile peanut oil were administered by subcutaneous injection. Observations on change in color were made once a week.

RESULTS. Groups of 6 to 8 male mice were kept on the deficient diet until they showed marked graying. Therapy then was instituted and observations continued for a further period of 2 to 16 weeks. Based upon this therapy, the animals are divisible into the five following groups:

Group A: 100 gammas of p-aminobenzoic acid in each milliliter of drinking water.

Group B: 70 gammas of calcium pantothenate and 350 gammas p-aminobenzoic acid in each milliliter of drinking water.

Group C: 70 gammas calcium pantothenate, 350 gammas p-aminobenzoic acid and 100 gammas inositol in each milliliter of drinking water.

Group D: 100 gammas calcium pantothenate, 100 gammas p-aminobenzoic acid and 100 gammas cystine in each milliliter of drinking water.

Group E: Rockland mouse diet and oats.

No change in the degree of achromotrichia was noted in groups A to D inclusively. The animals of group E regained their original hair color, but the

response was slow. An additional group of 9 mice (group F), kept on the deficient diet for 20 weeks, responded only partly to Rockland's fodder and oats, and some of the animals retained several light gray-brown spots for the rest of their lives. These observations seem to indicate a lasting damage to the melanin producing mechanism resulting from prolonged dietary deficiency.

It is also worthy of note that the C-57 strain was relatively resistant to the pantothenic acid deficient diet. More than half of the mice survived for four months or longer, while other strains are reported to succumb to the same deficiency within 8 to 10 weeks (10).

TABLE 1

DAY OF EXPERIMENT	TREATED WITH HORMONES			CONTROLS		
	Negative	Slight graying	Marked graying	Negative	Slight graying	Marked graying
A. Male						
26	10	5	—	12	2	—
35	7	7	1	2	6	6
47	4[a]	8[a]	1	—	4[a]	5[b]
54	2	9	2	—	2	7
61	1[c]	6	3[e]	—	—	7[d]
B. Female						
26	13	1	—	12	—	—
35	11	2	—	10	2	—
47	10	2	2	9	3	—
54	10	2	2	9	2	1
61	7[f]	3	2[f]	7[g]	1[h]	1

a–one mouse died on the 46th day.
b–four mice died on the 46th day.
c–one mouse died on the 59th day.
d–two mice died on the 59th day.
e–one mouse died on the 60th day.
f–one mouse died on the 58th day.
g–two mice died on the 58th day.
h–one mouse died on the 60th day.

Prevention of achromotrichia with p-aminobenzoic acid and/or pantothenic acid was studied in four groups of C-57 adult mice. Each group consisted of 8 males and 7 females fed the deficient diet supplemented as follows:

Group (1): 750 gammas of p-aminobenzoic acid in each milliliter drinking water.

Group (2): 100 gammas of calcium pantothenate and 750 gammas of p-aminobenzoic acid in each milliliter drinking water.

Group (3): 100 gammas calcium pantothenate in each milliliter drinking water.

Group (4): served as control and was kept on the deficient diet.

Graying was not prevented by the addition of p-aminobenzoic acid or calcium

pantothenate alone or combined. However, the sex differences previously reported (7) were observed. For this reason and the fact that Forbes (11) reported local pigmentation after subcutaneous implantation of pellets of various estrogens in Albino rats but no effect with testosterone dipropionate similarly used, another experiment was set up.

Each of 15 male mice received 1 mgm. of theelin (Lilly) on the first day and 0.5 mgm. theelin on the 12th and 21st day of the experiment. Fourteen female mice were injected with identical doses of testosterone dipropionate on corresponding days. All animals, including 14 males and 12 females that served as controls, were kept on the deficient diet. The results summarized in table 1 indicate that female sex hormones administered to male mice decrease the rate and degree of graying. Testosterone dipropionate seemed to have no influence on the development of achromotrichia in the female mice.

SUMMARY

Evidence is offered indicating involvement of endocrine factors in the achromotrichia that mice of the C-57 strain develop on a vitamin deficient diet.

The females are less susceptible to graying than the males.

P-aminobenzoic acid and pantothenic acid alone or combined do not prevent or cure graying in the C-57 strain of mice. Addition of inositol or cystine to the diet is without effect.

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(c) *"Alcoholic" extracts.* Extracts which were termed "alcoholic extracts" were prepared in the following way. The finely divided muscle was extracted 4 times with 80 per cent ethyl alcohol, after which negligible amounts of solids were removed from the tissue. The filtered extract was evaporated to a small volume under reduced pressure at low temperature. After suitable dilution, the "lipids" were removed by means of a continuous extraction apparatus, using ethyl ether as the solvent. The aqueous portion was then concentrated under reduced pressure and the extract volume was adjusted so that 1 ml. of extract was equivalent to 7 grams of the original muscle. Only very small amounts of nitrogenous material could be obtained from this extract by precipitation in 5 per cent trichloroacetic acid, so that it may be considered to be practically protein free. The extract was also free from "lipid" material. As shown in the tables, considerable amounts of non-protein nitrogenous material and ash were present.

To determine the organic and inorganic solids of the extracts, the total dry weights were first obtained by drying at 110°. These solids were then ignited in a muffle furnace and the weight of the ash found. The potassium procedure adopted was similar to the chloroplatinate method used by Fenn et al.

RESULTS. 1. *Toxicities of the Muscle Extracts.* In table 2 a comparison is given of the toxicities of extracts made from normal and traumatised muscle. The protein solution had no effect on normal mice although it produced some deaths in adrenalectomised rats. Its toxicity was slight in relation to the large amounts of organic solids administered. Since none of the test animals had received injections prior to the experiments, the reactions were not attributed to sensitivity to foreign proteins. The saline and water extracts were only slightly toxic, due probably to the fact that the solutions were not concentrated, and hence the muscle equivalent of the amount injected was low.

The "alcoholic" extracts which were readily concentrated were more toxic than the above solutions. In adrenalectomised rats an injection of extract containing 50 to 100 mgm. of organic solids and 15 to 35 mgm. inorganic solids was rapidly fatal in most of the animals, while in normal mice 107 to 117 mgm. of organic solids and 41 mgm. of inorganic solids were fatal to most. "Blank" extracts using the reagents alone showed that the toxicity was not due to substances contained in them. The osmotic pressure of these "alcoholic" extracts was determined by the depression of the freezing point, after suitable dilution of the solutions. The osmotic pressures of extracts from traumatised and normal muscle were found to be equivalent to 1.86 and 1.93 M solutions of non-electrolyte respectively. It was thought that this high osmotic pressure might be the cause of the lethal effects. Aqueous solutions of sodium chloride and of urea of approximately equivalent osmotic pressures were therefore injected into test animals. These solutions were found to be non-toxic, so that the lethal effects could not be entirely attributed to the high osmotic pressures of the solutions. Generally the "alcoholic" extracts were diluted 3-fold and given in divided doses. This method of administration produced slightly less toxic effects than a single dose of the original solution.

It should be emphasized that in these experiments no significant difference could be observed between the toxic effects of extracts of traumatised muscle and those of normal muscle.

2. *Toxic Substances in the "Alcoholic" Extracts.* (a) *Histamine.* The histamine content of the alcoholic extract, determined[1] by the method of Best and

TABLE 2

The effects of intraperitoneal injections of extracts from muscle on the survival of adrenalectomised rats and normal mice at temperatures of 15°C and 22°C, respectively

ANIMALS	BODY WEIGHT AVERAGE	MUSCLE EXTRACTED	SOLUTION INJECTED	MATERIALS INJECTED*			NUMBER OF ANIMALS	NUMBER OF SURVIVALS
				Equivalent of muscle	Organic solids	Inorganic solids		
	grams			*grams*	*mgm.*	*mgm.*		
Adrenalectomised rats	102	Traumatised	Muscle protein	6	116	3.4	7	5
	100	Normal	Muscle protein	6	119	0.9	7	5
	117		Saline				9	8
	51	Traumatised	"Alcoholic" extract	4	50	15	5	1
	115	Traumatised	"Alcoholic" extract	6	92	35	6	1
	58	Normal	"Alcoholic" extract	4	72	25	5	1
	122	Normal	"Alcoholic" extract	6	101	35	6	1
	76		Saline				5	5
	120		Saline				6	5
Normal mice	24	Traumatised	Muscle protein	5	80	3.3	6	6
	24	Normal	Muscle protein	5	116	1.5	6	6
	24		Saline				6	6
	25	Traumatised	"Alcoholic" extract	7	107	41	24	9
	25	Normal	"Alcoholic" extract	7	117	41	24	1
	23		Saline				20	20

Control animals were given saline instead of solutions prepared from muscle.

* The figures referring to rats are expressed in grams or milligrams per 100 grams of body weight, those referring to mice in grams, or milligrams per animal.

McHenry, was found to be 0.16 to 0.17 mgm. histamine per 100 grams of the original tissue from normal and injured muscle respectively. The dose of histamine which proved lethal to adrenalectomised rats was about 15 mgm. per 100 grams body weight. The corresponding dose of "alcoholic" extract per 100 grams body weight was equivalent to the extract obtained from 6 grams of muscle, and this amount of extract contained only 10 micrograms of histamine. It is evident

[1] We are indebted to Dr. E. W. McHenry for the histamine assays.

that under the conditions of the test, the toxicity of this extract was not due to histamine.

Since guinea pigs are relatively very sensitive to histamine poisoning, tests of the toxic effects of the extracts were made in these animals. Injections of about 3 mgm. of histamine per 100 grams body weight proved toxic to 50 per cent of the guinea pigs. The corresponding dose of "alcoholic" extract was equivalent to the extract from 8 grams of muscle, which contained 14 micrograms of histamine. Thus, even when tested on guinea pigs, the histamine of the muscle extract had no appreciable toxic effect.

TABLE 3

Survival of normal mice given intraperitoneal injections of "alcoholic" extracts of normal muscle, the ash of the extracts and equivalent amounts of potassium chloride in solution

BODY WEIGHT AVERAGE	SOLUTION INJECTED	AMOUNT OF KCl INJECTED	NUMBER OF ANIMALS IN GROUP	NUMBER OF SURVIVALS
grams		*mgm.*		
23	"Alcoholic" extract*	39.1	18	2
22		29.3	18	3
21		19.5	18	9
21		9.8	18	18
21		3.9	18	18
21		0	18	18
22	Ash of the "alcoholic" extract*	39.1	18	0
22		29.3	18	1
21		19.5	18	1
21		9.8	18	17
22		3.9	18	18
21		0	18	18
22	Potassium chloride 4% solution	40	12	0
21		30	12	0
22		20	12	1
21		10	12	12
21		4	12	12
21		0	12	12

* The potassium concentration was determined by actual analysis.

(b) *Choline.* Tests on the "alcoholic" extract, according to a slight modification of the method of Jacobi, Bauman and Meek (1941), failed to reveal the presence of significant amounts of free choline. This negative finding is in agreement with the work of others (Guggenheim, 1940).

The dose of choline chloride which proved fatal to 50 per cent of the adrenalectomised rats injected was about 70 mgm. per 100 grams body weight. In mice the corresponding dose was 10 mgm. per 100 grams body weight. The amounts of "alcoholic" extract required to produce these effects corresponded to extract from 4 grams and 2.8 grams of muscle respectively. Hence the toxicity of the ex-

tracts was not due to choline. Further experiments showed that neither the creatine nor urea content of the extract was responsible for the injurious effects.

(c) *Potassium.* Since the toxic material could be dialysed from the "alcoholic" extract through collodion membranes, the ash of the extract was examined for toxicity. The extract was dried and ignited in a muffle furnace and the ash obtained was then dissolved in dilute hydrochloric acid and the solution adjusted to pH 7. This solution was toxic. By comparing the effects produced by graded amounts of the "alcoholic" extract with those of aliquots of the extract ash, it was demonstrated (table 3) that all the toxicity of the alcoholic extract was due to inorganic solids. Potassium was first investigated as the most likely cause of these effects. The ash in the extract from 100 grams of muscle was found to contain 293 mgm. of potassium which is equivalent to 560 mgm. of potassium chloride. The total potassium chloride to be expected from 100 grams of dog muscle is about 745 mgm., so that the extraction procedure removed approximately 75 per cent of the total potassium. The total ash extracted amounted to 786 mgm. per 100 grams of muscle.

TABLE 4

Levels of the potassium in the blood plasma of mice following lethal doses of potassium chloride solution, and of "alcoholic" extracts of normal muscle

BODY WEIGHT AVERAGE	NUMBER OF ANIMALS IN GROUP	SOLUTION INJECTED	AMOUNT OF KCl INJECTED	PLASMA POTASSIUM
grams			*mgm.*	*mgm. %*
31	6	"Alcoholic" extract	39.1	75.8
30	7	"Alcoholic" extract	39.1	91.0
31	5	Potassium chloride solution	40	100.5
29	5	Potassium chloride solution	40	83.2
32	8	No injection	0	35.0
31	6	No injection	0	31.9
30	4	4% NaCl	0	40.3
	3	No injection	0	24.3*

* The potassium content of the heart blood plasma of normal unanesthetized mice in which greater care was taken to avoid bleeding to death than in any of the other groups.

The toxicities of a series of aqueous solutions of potassium chloride were then tested on adrenalectomised rats, normal mice and guinea pigs. The results (table 3) showed clearly that potassium was toxic under the conditions of the test and that the number of animals which succumbed depended on the amount of potassium injected. The results also showed that the toxicity of the extract ash could be entirely accounted for on the basis of the potassium content. There is little doubt that the toxicity of the "alcoholic" extracts of both normal and traumatised muscle was due to the potassium contained in them.

In order to obtain further justification for this conclusion, the potassium concentrations of the blood plasmas were determined in adrenalectomised rats,

normal mice and guinea pigs which had received the test injections. The animals were stunned and the blood obtained by heart puncture as soon as the first signs of poisoning were observed. The results on mice are recorded in table 4. The mice were given 1 ml. of extract which was equivalent to 7 grams of muscle and which contained 39.1 mgm. of potassium chloride. Similar amounts of the salt were injected in 1 ml. of a 4 per cent potassium chloride solution. As the table shows, these caused an increase in plasma potassium which is quite incompatible with life. Winkler et al. found a concentration of 50–60 mgm. per cent to be the lethal concentration for dogs. Attention is drawn to the concentration of 35 to 40 mgm. per cent found in uninjected mice or those given saline. This is usually high for mammalian blood plasma, but it is a suitable control for the other groups since the mice were all handled in an identical fashion. In the last group listed in table 4, greater care was taken in handling the animals as well as in avoiding bleeding to death. It is of interest that this improved procedure caused the potassium value to fall to concentrations which are more reasonable for normal mammalian plasma.

3. *Effects on Blood Pressure.* The effects of certain of these solutions on the arterial blood pressure of the dog were noted. The solutions were given intravenously to dogs under ether anesthesia and the blood pressure in the carotid artery was recorded. The "alcoholic" extracts produced a definite fall in blood pressure of which part must have been due to the small amounts of histamine present. Only minor effects were caused by the protein extracts. The solutions containing aliquots of the ash from the "alcoholic" extracts produced relatively slight or negligible reductions in blood pressure. Solutions containing equivalent amounts of potassium chloride injected at the same rate also produced only slight effects. This indicates that the lethal effects of tissue extracts are not necessarily correlated with substances causing a fall in blood pressure. The investigations of Green (1943) deal with similar aspects of these problems.

SUMMARY

It is fully realized that the relative toxicities of the various components of muscle extracts vary widely with different test animals. However if rigid experimental conditions are maintained, adrenalectomised rats, normal mice and guinea pigs can be used to test for the toxic effects of various solutions.

Certain "alcoholic" extracts of traumatised or normal dog muscles were found to cause death under the conditions of the test. Muscle protein solutions were much less toxic. No significant differences between the toxicities of extracts from traumatised or normal muscles were found.

The toxic factor was found in the ash of the extract and was identified as potassium. Since many tissues contain a relatively high concentration of potassium, the possibility should not be overlooked that it might produce a toxic effect when tissue extracts are used to produce shock. The effects of these solutions on the blood pressure of dogs were noted.

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THE EFFECT OF MEASUREMENT TECHNICS ON THE VALUES FOR RED CELL DIAMETER, WITH SOME OBSERVATIONS ON THE RELATIONSHIP BETWEEN CELL DIAMETER AND OTHER FACTORS IN THE BLOOD PICTURE[1]

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Received for publication January 17, 1944

The normal size of the red blood cell and the significance of deviations from the normal have received considerable attention in recent years. In consequence, physiological factors which influence cell diameter, as well as those inherent in the measurement technic employed which affect the values obtained, become of definite importance. A variety of methods for measuring cell diameter have been used by investigators. Among these is the Price-Jones (1) technic in which the projected images of the red cells in a dry film are measured. Additional methods which have received wide acceptance are: the use of the ocular micrometer for the determination of the diameter of red cells in either a dry or a moist film; and the measurement of cells in a photomicrograph (2).

Workers disagree as to the effect of the method of preparing the film on red cell diameter values. Some investigators (1, 3, 4) obtained a decreased value when a dry film was used, others (5, 6) noted an increase, while still others (7) observed no change. Jørgenson and Warburg (6) suggested that the high values secured when a dry preparation was used resulted from the measurement of diffraction bands surrounding the cells as a part of the diameter. These bands they reported as nearly 0.6 micron wide.

Another approach to the estimation of cell size is that of mean corpuscular volume determinations. Several workers (8, 9) have pointed out that cell diameter and mean corpuscular volume are independent characteristics, while others (10) have indicated that cell volume can be taken as an index of cell size in certain forms of anemia, but not in others.

The purpose of the present study was to establish the extent of the differences in cell diameter attributable to certain factors in the technic of measurement and to determine the relationship between the diameter and the volume of the red cell in normal young women.

PROCEDURE. To determine the magnitude of the difference in the values obtained from measurements made on wet and dry films, parallel series of 50 blood films were prepared and one diameter of 200 round cells was measured on each preparation using the 4 mm. objective of a Bausch and Lomb microscope and a calibrated filar micrometer. For each blood sample two dry films were prepared and 100 cells were measured on each. In an earlier study (11) in this laboratory

[1] Approved for publication by the Advisory Committee as Paper no. 22 of the Regional Project of the North Central States relating to the Nutritional Status of College Women.

Paper 2136, Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul.

it was demonstrated that the mechanics of spreading the drop was without influence on the shape of the cell and hence one diameter was considered an adequate measure of cell size. Wright's stain was used in the preparation of all dry films. An additional series of 33 dry blood films was measured, first using the 4 mm. objective and then the oil immersion lens.[2] Since the two series were carried out on different subjects with somewhat different mean cell sizes, as shown by the values obtained on the dry films, it was not possible to make a direct comparison of the results obtained. Consequently, a third series of measurements on eight blood samples was made, employing the three technics.

To establish the relationship between cell diameter and mean corpuscular volume in normal subjects, parallel measurements were made on a series of 260 blood samples from college women. For the mean corpuscular volume measurements, the Van Allen hematocrit was employed using heparin as the anticoagulant, since it had been shown in an earlier study (12) that salts caused some

TABLE 1

Comparison of red cell diameter values using three methods of measurement (in microns)

SAMPLE NUMBER	MOIST FILM 4 MM. OBJECTIVE	DRY FILM 4 MM. OBJECTIVE	DRY FILM OIL IMMERSION OBJECTIVE
1	7.581	7.664	7.214
2	7.718	7.873	7.210
3	7.527	7.681	7.078
4	7.478	7.886	7.247
5	7.705	7.885	7.369
6	7.464	7.609	7.004
7	7.553	7.788	6.932
8	7.378	7.592	6.860
Mean.....	7.550	7.747	7.114

alteration in cell size as compared with the values obtained when no anticoagulant was used, whereas heparin was without effect.

RESULTS AND DISCUSSION. The measurements in the first series gave a mean diameter for the cells in the wet films of 7.62 microns and for the cells in the corresponding dry films of 7.90 microns, a mean difference of 0.28 micron. The mean of the diameter values on the dry films secured in the second series was 8.14 microns as compared with 7.59 microns when measurements were made using the oil immersion objective, a mean difference of 0.55 micron. This difference is of essentially the same magnitude as that of the diffraction bands as reported by Jørgenson and Warburg.

The results in series 3, given in table 1, show a mean of 7.55 microns for the cells in the moist film, of 7.75 microns for the cells in the dry film and of 7.11 microns for the cells in the dry film measured with the oil immersion objective. From this it is apparent that the technics of film preparation and of measurement are factors of considerable importance, and cognizance of the particular method employed is essential in the interpretation of the results obtained.

[2] Conversion factor for the 4 mm. objective, 0.2092; for the oil immersion objective, 0.0939.

The coefficient of correlation secured between the red cell diameter and mean corpuscular volume values obtained on the 252 blood samples indicated a fairly high degree of relationship between these two cell characteristics ($r = 0.4606$, $P = < 0.0001$). As a further test of this relationship, table 2 was prepared showing the mean cell diameter which corresponds to a given range in mean corpuscular volume. It is evident that increases in cell volume are paralleled by a practically uniform increment in diameter. The magnitude of the standard errors indicates the variation in mean diameter which occurred within a given range of mean corpuscular volume.

In an earlier study on college women (13), a significant increase in mean cell diameter was noted when measurements were made on the same individual at six-month and yearly intervals. To determine whether such changes in mean cell diameter are associated with corresponding changes in mean corpuscular volume, a series of cell diameter and mean corpuscular volume measurements were made on 54 subjects. After an interval of one year the determinations were repeated.

TABLE 2

Distribution of mean cell diameter measurements for a given range of mean corpuscular volume

NUMBER OF CASES	MEAN CORPUSCULAR VOLUME		CELL DIAMETER	
	Interval	Midpoint	Mean	Standard Error
	cubic microns	*cubic microns*	*microns*	*microns*
16	75.0–79.0	77.5	7.27	0.16
54	80.0–84.9	82.5	7.30	0.18
76	85.0–89.9	87.5	7.35	0.20
55	90.0–94.9	92.5	7.40	0.22
39	95.0–99.9	97.5	7.51	0.16
15	100.0–104.9	102.5	7.55	0.09
5	105.0–109.9	107.5	7.60	0.04

At the beginning, the mean diameter for the 54 subjects was 7.41 microns and the mean corpuscular volume, 87.0 cubic microns. One year later the values were 7.59 microns and 96.7 cubic microns, respectively, representing increases of 0.18 micron in diameter and 9.7 cubic microns in volume. Both increases were found to be highly significant ($P = < 0.0001$).

To test the relationship between cell size and other blood measurements regularly used in clinical diagnosis, coefficients of correlation were computed between cell diameter and red cell count, between diameter and hemoglobin, and between mean corpuscular volume and hemoglobin on data from 252 women students. The results indicate a significant inverse relationship between cell diameter and red cell count ($r = -0.2978$, $P = < 0.0001$), from which it is clear that individuals who have a high cell count tend to have cells of a smaller size. In these data on normal subjects, no relationship of statistical significance was observed between hemoglobin and cell diameter or between hemoglobin and mean corpuscular volume.

SUMMARY

Diameter values for red blood cells are highest when measurements are made on a dry film with a 4 mm. objective, somewhat lower, on a moist film and lowest when measurements are made on a dry film with an oil immersion lens.

A highly significant relationship exists between the diameter and the volume of the normal red blood cell.

High red cell counts are associated with cells of relatively small size in normal blood samples, as shown by a significant negative coefficient of correlation between cell count and cell diameter.

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A STUDY OF LEG ANOMALY CAUSED BY CONFINING CHICKENS IN SMALL CAGES[1]

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Received for publication January 10, 1943

Chickens raised individually in small cages for the purpose of investigating certain nutritional and disease problems developed leg weakness which culminated finally in rotation of the leg bones and displacement of the Achilles tendon over the condyle (fig. 1). An apparent shrinkage or wasting of the soft tissues of the shanks as indicated in figure 2 usually accompanied the disorder. Inasmuch as close confinement of birds is frequently necessary in certain types of research, it was deemed worthwhile to investigate the cause of this anomaly.

Chickens, confined as described, developed this syndrome if fed practical poultry rations that had been used satisfactorily for years. Birds raised in batteries or on the floor and fed these same rations did not develop this condition. Administration of manganese, dried yeast, dried liver, and vitamin D failed to restore these birds to normal condition; in fact, in exploratory experiments, supplements of α-tocopherol, riboflavin, vitamin D, choline, thiamine, calcium pantothenate, vitamin B_6, gelatin, and liver meal added to the ration from the beginning of confinement failed to prevent this abnormality. It occurred when birds were on wire floors of large and small mesh.

Inasmuch as leg disorders have also been attributed to disease, post-mortem examinations were always made and tissues taken for histological study. Microscopic lesions of lymphomatosis were present to a degree previously unrealized in such young birds but this disease was not the causal factor of the abnormality as will be brought out later. Because atrophy of disuse occurs in animals that are deprived of normal exercise, it was thought that this might account for the wasting of tissue and weakness of the legs. Since histological studies of the muscles failed to show changes characteristic of disuse atrophy, it was decided to study the fatigability or the capacity of the muscles of birds reared under these conditions to do work.

EXPERIMENTAL. *Confinement.* Chicks in lots I and III were given the freedom of a double section of a Bussey battery brooder which measured 3 by 6 feet, providing 18 sq. ft. area for their activity. These are termed "battery" birds in the following discussion. Those in lots II and IV were closely confined individually in small isolation cages which furnished them a space 17 by 7.5 inches or 0.9 sq. ft. These are called "cage" birds. The space allowed seemed ample for normal activity up to about 6 weeks of age; movement after this time became increasingly restricted. On the other hand, the "battery" birds were confined to a degree common in practical battery brooding. All birds were

[1] Michigan Agricultural Experiment Station Journal Article no. 676 (n.s.).

raised indoors. The windows were open but the birds were not directly irradiated.

Rations. Lots I and II received ration A; lots III and IV, ration B. Both rations, as previously mentioned, were practical poultry starting rations used for the flocks at the institutions involved and made up of the usual natural feed ingredients. The rations differed only slightly in composition; ration A had more calcium and vitamins A and D. When fed to chickens either in batteries or on the floor, they gave satisfactory results. Water and feed were before the birds at all times.

Source and age of chickens. Six different pedigreed matings of Single Comb White Leghorns contributed one chick to each lot from three hatches, 3 weeks apart. Thus, heredity as a possible variant was controlled as much as possible. Chicks were one day old when placed on experiment. Birds were removed from a particular lot for physiological testing and autopsy as soon as leg weakness appeared and, at the same time, its sib was removed from the control lot. The remainder were tested at 8, 11 or 14 weeks when the experiment was concluded.

Fig. 1 Fig. 2

Fig. 1. Chicken raised in a small cage showing a severe case of leg anomaly

Fig. 2. The legs of the "cage" bird at the left show wasting of the tissues, giving an "angular" or "square" appearance to the shank. The legs of the "battery" control bird on the right are normal.

Measurements of fatigability. Each bird was weighed and anesthetised with approximately 0.35 ml. of Halatol[2] per kgm. of weight given intravenously. The lateral portion of the gastrocnemius muscle was dissected out and the smaller or medial portion separated from it. The tendon of the lateral portion was freed and attached to a pulley by means of a double linen thread. The bird was tied on its back to an adjustable platform so that the tibia was maintained in a perpendicular position. The distal end of the tibia was firmly held in position by flexing the tibial-metatarsal joint over a bar and clamping it in this position. The linen thread was attached to a pulley (16 mm. radius) on a shaft using sufficient tension to bring the muscle back to its normal position. The direction of pull when the muscle contracted was kept constant. To the same shaft was attached a pen (145 mm. in length) which wrote on glazed paper on a kymograph and a second pulley (18.5 mm. radius) which applied a load of 1.5 times the weight of the bird.

Fine copper wire electrodes attached to the secondary of an inductorium were

[2] From Jensen-Salsbery, Kansas City, Missouri.

inserted in the upper and lower ends of the muscle. The secondary was adjusted to the position giving maximum contraction with minimum current in single trials. The stimulus was then applied for 3 minutes with a tetanizing current having a frequency of 90 per second interrupted in such a manner that 0.5 second periods of stimulation alternated with similar periods of quiescence. To mark the end of each minute, the stimulus was interrupted for 1 second.

The area indicating the work performed in the first 30 seconds by the gastrocnemius of the weaker bird of the pair, consisting of the "cage" bird plus its battery sib, was measured on the graphs by means of a planimeter and computed to gram centimeters per second per kilogram of body weight. The time required for the stronger bird to do the same amount of work as the weaker one did in 30 seconds was found. The rate of work during 2.5 minutes following this period of equal work was obtained for both birds. This procedure was adopted because it was noted that "cage" birds frequently performed work satisfactorily at the start but fatigued more rapidly. The time in seconds and the length of contraction of the muscle at the end of the initial periods of equal work were also obtained and used as bases for comparing fatigability.

Autopsies. Following the fatigability tests, the birds were killed and examined for gross pathological lesions and specimens of gastrocnemius muscle, heart, liver, kidney, gonad, duodenum, pancreas, sciatic nerve, brachial plexus, adrenal ganglion and adrenal gland were fixed in formalin and embedded in paraffin. Sections were stained with hematoxylin and eosin for histological study. Additional muscle tissue was fixed in Zenker's fluid and stained with Mallory's triple stain. Nerve tissue was also fixed in ammoniated (1 per cent) absolute alcohol and stained with Ransom's pyridine-silver technic.

RESULTS. A summary of the results is given in table 1.

Gross symptoms. The "cage" birds weighed less than those brooded in the battery. While no records of feed consumption were kept, the former appeared to eat less feed. Whether inactivity or the lack of example of other birds eating caused this is not known.

The legs of the "battery" birds (lots I and III) appeared normal in all respects. Those of the "cage" birds (lots II and IV) showed varying degrees of wasting of the soft tissues, giving them an angular or square appearance (fig. 2). In addition, leg deformities as indicated by bowing of the legs or rotation of the bones with slippage of the tendon (fig. 1) were observed in 12 of 18 birds of lot II, and 11 of 18 in lot IV.

Muscle solids. Because the shanks of "cage" birds presented a wasted appearance, it was decided to find out if these tissues were dehydrated. Since the muscle is a sensitive indicator of dehydration, the moisture content of the gastrocnemius was obtained by drying to a constant weight at 105°C. "Cage" lots had an average of 77.75 per cent water; "battery" lots, 77.70 per cent. The shank condition, therefore, could not be explained on the basis of general dehydration.

Pathology. Histological examination of the tissues showed that 8 of the "cage" and 13 of the "battery" birds had microscopic lesions characteristic of lympho-

matosis. Inasmuch as the "battery" birds had a higher incidence of this disease, yet showed no gross symptoms of the leg abnormality, the latter could not have resulted from lymphomatosis. Furthermore, "cage" birds having definite cases of the leg disorder were not always affected with lymphomatosis.

Histological studies failed to show reduction in size of the muscle fibers and the atrophy or degeneration within the fibers which are characteristic of atrophy of disuse. There was no degeneration of the nervous tissue. Other tissues examined were also normal except for the lymphomatosis described.

Fatigability. As an illustration of the type of data obtained, the protocol on one set of birds follows: Birds X4, Y4, Z4, and A5 were hatched from mating

TABLE 1

A comparison of birds raised in batteries with those raised individually in cages

	RATION A		RATION B	
	Battery	Cage	Battery	Cage
Number of birds	15	18	14	18
Birds with leg anomaly symptoms	0	12	0	11
Birds with microscopic lesions of lymphomatosis	5	3	8	5
Age in days when tested (av.)	71	65	73	74
Weight in grams when tested (av.)	880	750	960	930
Per cent H_2O in gastrocnemius muscle (av.)	77.7	77.7	77.7	77.8
Average rate of work (gm. cm./kgm. body wt. during 2.5 min. following period of equal work)	397	245*	400	265*
Average rate of work (gm. cm./sec./gm. dry gastrocnemius muscle during 2.5 min. following period of equal work)	252	149**	256	166**
Contraction at end of period of equal work as percentage of original contraction (av.)	49.3	30.5*	52.8	26.9**
Time in secs. required for the stronger bird of a pair to equal work of weaker one in 30 secs. (av.)	23.9	28.1*	21.7	28.7**
Gastrocnemius muscle (wet) as percentage of body wt. (av.)	0.73	0.68*	0.70	0.71

*, ** Value of t (Fisher) of the difference between battery and cage lots exceeds the 5 and 1 per cent point of significance, respectively.

758 and placed in lots 1, 2, 3, and 4 respectively. X4 and Y4 were fed ration A; Z4 and A5, ration B. At 50 days, A5 showed definite rotation of the legs; therefore, it and its control in the battery were removed at this time for testing. X4 did not develop definite clinical symptoms and was not tested with its control until 87 days, at which time the experiment was terminated.

Bird Z4 weighed 667 grams and, therefore, was given a load of 1000 grams to lift; A5 weighed 760 grams and was given 1140 grams. They were prepared for testing as described and their ability to perform work is shown in figure 3. A5 fatigued rapidly, later recovered slightly. On the other hand, Z4 fatigued more slowly and was able after a short time to perform a certain amount of work continuously for the period studied.

The work performed (area) during the first 0.5 minute for A5 was measured by the planimeter. By trial, an initial area of equal size was found for Z4 and from this the time required for equal work (abcissa) was obtained. The rate of work done in the 2.5 minutes following these periods of equal work was measured for both birds. The height to which the load was lifted at the end of the preliminary periods of equal work (contractability) was measured in millimeters for both birds by curving a celluloid rule along the arc.

The effect of confining birds to cages is observed from the data in table 1. The rate of work after initial periods of equal work by the gastrocnemius muscles of "cage" birds fed both rations was considerably less than that of "battery" birds. For example, the work per second per "cage" bird averaged only 255 gm. cm. per kgm. body weight compared with 398 for those raised in the battery.

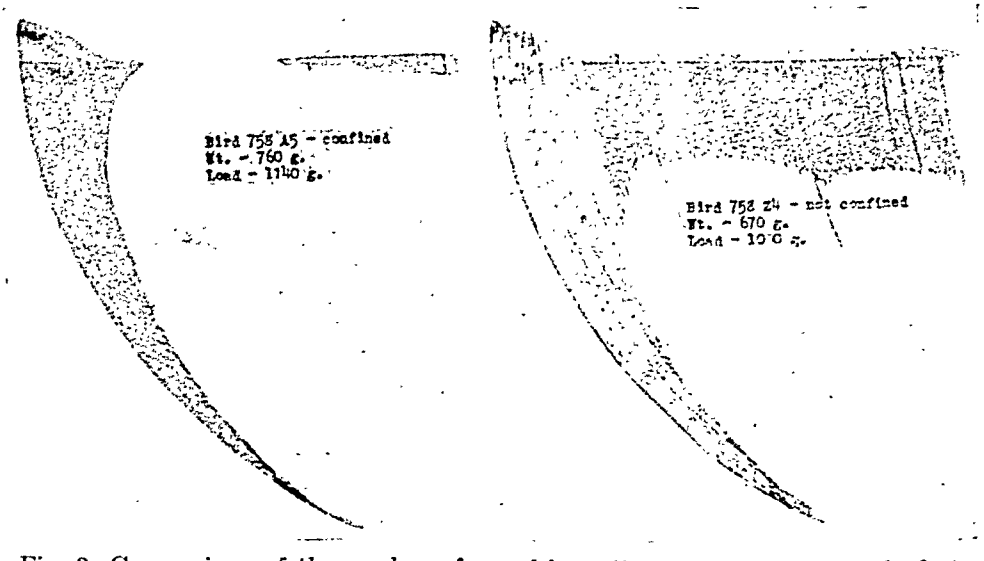

Fig. 3. Comparison of the work performed by a "cage" bird (left) with that of its sib raised in a battery.

The length of contraction of the gastrocnemius after doing an amount of work equal to that performed in 30 seconds by the weaker of the paired chickens was 28.7 per cent of the original contraction in the "caged" birds compared to 51.1 per cent for those in the battery.

"Cage" chickens required 28.4 seconds to perform the same amount of initial work as was done by the "battery" birds in 22.8 seconds.

These differences between the "cage" and "battery" chickens are statistically highly significant. Very close confinement in cages, therefore, caused birds to develop a leg-muscle weakness which led finally to definite leg deformities.

Effect of age. As previously stated, as soon as a bird in a particular lot showed clinical symptoms, it and its sib in the control lot were removed, tested, and autopsied. Consequently, birds were not of the same age in all the lots. In each lot, there were also birds of three different hatches, 3 weeks apart. Statistical analysis of the data shows, however, that the age at which birds were tested did

not affect significantly the ability to do work either between lots on different rations or between those confined in cages or in the battery.

Effect of sex. The number of males in the different lots varied from 8 to 12 and of females from 6 to 10. In the "battery" lots, males did more work per sec. than the females (110 gm. cm./sec./kg. body weight). This apparent variation did not affect the significance of the difference between "cage" and "battery" lots.

Effect of ration. The work capacity of lots which were fed the two rations A and B were not significantly different. This was true whether birds were brooded in either batteries or cages.

DISCUSSION. The data presented indicate that very close confinement in cages rather than inadequate nutrition, disease, or other management practices was responsible for the leg anomaly described. The birds were caged as individuals but the condition has been observed previously if one or two birds were placed in a cage twice the size as the ones used in this study. Possibly lack of competition for feed, therefore, is not such an important factor in the problem.

The data suggest that the use of small cages created a disturbance in normal muscle physiology prior to the development of gross abnormalities. If inactivity were the causal factor, then atrophy or degeneration should have been evident. Yet, the lack of any muscle tissue changes does not support a diagnosis of disuse atrophy, as described for other species (2). If malnutrition or partial inanition were the indirect result of caging, a reduction in the diameter of the muscle fibers would have been expected. It has been reported, however, that muscle fibers show no decrease in diameter in severely malnourished infants (1). Aqueous inanition in chickens causes the skeletal muscle to show occasional slight hemorrhages, with partial loss of striation (1). The interstitial stroma presents a round cell infiltration. In animals deprived of water, the musculature may lose amounts of water easily measured by chemical analysis. None of these were found in the present experiment.

Birds naturally infected with lymphomatosis usually show gross symptoms in about 16 to 24 weeks. It was, therefore, surprising to find evidences of this disease in birds as young as 50 days. This finding is of considerable importance because many birds are used for research at this early age, frequently without post-mortem histological examination being made.

SUMMARY

Chickens isolated in small cages developed leg weakness and, finally, leg deformities. In the terminal stages, the leg bones were deformed and the Achilles tendon slipped over the condyle. Wasting of the leg tissue usually accompanied the disorder.

When compared with chickens brooded in a battery, the birds raised in small cages were inferior in the capacity of their gastrocnemius muscles to perform work. There was no evidence of dehydration of the muscle tissue.

Nutrition and other phases of management were not related to the development of the anomaly.

Lymphomatosis was detected by histological examination to some extent in all lots of birds, hence it did not appear to be the causal factor of this anomaly. The finding of lesions of lymphomatosis in such young stock was considered of particular interest.

A method was developed for measuring the comparative fatigability of the gastrocnemius muscle of chickens when stimulated by a tetanizing current. Criteria for comparison were the time required to do equal amounts of work, the rate of work performed in a definite interval, and the height to which a load could be lifted after this initial period of equal work.

We wish to thank Director Berley Winton and Dr. N. F. Waters of the Regional Poultry Research Laboratory for their aid and for supplying the pedigreed chicks used in the investigation; also Dr. W. D. Baten of the Michigan Agricultural Experiment Station for his helpful advice on statistics involved.

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PEPSIN SECRETION AND ENTEROGASTRONE

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Received for publication January 10, 1944

The effect on the secretion of pepsin of fractions of the upper intestinal mucosa having considerable enterogastrone or gastric secretory inhibitory activity constitutes a problem which has not hitherto been investigated. The existing evidence indicates that stimulation of endogenous enterogastrone production in the animal body, as by the oral administration of fat, is accompanied by inhibition of pepsin secretion (1–8). Inasmuch as fat depresses all moieties of gastric activity, i.e., motility, volume of secretion, acid concentration and output, and pepsin concentration and output, and, since it has been demonstrated (9, 10) that motor inhibition primarily or entirely and volume and acid inhibition at least in part are effected by humoral or hormonal agencies, it might be anticipated that the inhibition of pepsin secretion by fat is also accomplished by a humoral or hormonal mechanism.

The routine assays of enterogastrone preparations in our laboratory have shown that such preparations decrease the total output of acid more than the volume output of fluid. Thus the concentration of acid is reduced as well as the volume of secretion, resembling in this respect the effect of fat administration. Hence it was deemed pertinent to determine whether parenterally injected enterogastrone, like fat, also decreases pepsin secretion.

This question has definite practical implications, as it has been shown that an enterogastrone concentrate prevents the development of ulcer in Mann-Williamson dogs (11) and that artificial gastric juice (HCl-pepsin solution) is more irritating to the intestinal mucosa than dilute HCl having a similar pH (12–14).

It has been reported (15) that the depression of volume output and pepsin concentration produced by fat is dependent upon the integrity of the vagus nerves. It has since been demonstrated (10) that the inhibition of volume and acid output is at least in part accomplished by a humoral or hormonal agency. In order to secure a delineation of the roles of humoral and nervous components it was necessary to investigate the effect of enterally administered fat and parenterally administered enterogastrone on pepsin secretion in vagal denervated and vagal innervated gastric pouches.

It was further necessary to reexamine the possibility that a reduction in the acidity of gastric juice inactivates pepsin. Agents which inhibit the secretion of acid were to be used so that an observed decrease in pepsin output might be due to inactivation rather than to diminished secretion. In this connection one report (16) indicates that pepsin is most stable at pH 5.0, whereas another (17) states that pepsin is appreciably inactivated at pH's greater than 1.3. Although the reported disagreement may have been due to differences in the pep-

sins studied, it was believed probable that the discrepancy might be caused by the technique used.

METHODS. *The determination of pepsin.* Gastric juice samples were diluted with 0.01N HCl and the pepsin content determined by the method of Anson and Mirsky (16) using the Klett-Summerson photo-electric colorimeter. The dilutions used for pilocarpine and histamine stimulated juice were 1:49 (1 part of juice and 49 parts of 0.1 N HCl) and 1:24 respectively. The results are expressed in U^{hb} (hemoglobin units) $\times 10^4$ as defined by Anson (16).

The question of the inactivation of pepsin at pH's up to approximately 6 was studied by two methods: (A) Samples of canine gastric juice were diluted 1 to 9 with appropriate acetic acid-sodium acetate buffer solutions to attain a series of pH values between 1 and 6. The pH of the resulting mixture was determined by the use of the glass electrode. The pepsin content was compared with that of a similar dilution with 0.1 N HCl. (B) Samples of canine gastric juice were diluted 1:24 with 0.1 N (pH 1.02), 0.01 N (pH 2.00), and 0.001 N (pH 3.00) HCl and the peptic activity of the diluted juice determined

Experimental animals and test procedure. Histamine and pilocarpine were employed as stimulating agents in the dogs described below and were injected subcutaneously after noting that the secretion from the stomach was at a basal level as indicated by the absence of free acid. The dosage of histamine varied according to the response of the individual animals but was always the same for any given animal. All dogs received 3 mgm. of pilocarpine hydrochloride. The acid content of the gastric juice was determined by titration and is expressed in milligrams of HCl.

Series I. Seven dogs with vagal denervated pouches of the entire stomach and 2 dogs with vagal innervated, large isthmus, greater curvature (Pavlov) pouches were used in this study. Two of the former group were also provided with subcutaneously transplanted pouches. Following stimulation, the gastric juice was collected for 90 minutes. After this collection a rest period of 90 minutes was allowed; the identical stimulus was then repeated preceded by either the instillation of 100 cc. of 50 per cent olive oil emulsion by stomach tube an hour before the second injections of the stimulus or the intravenous injection of 50 milligrams of enterogastrone concentrate 20 minutes beforehand. In the control experiments no inhibitory procedure preceded the second stimulation. Gastric juice was again collected for 90 minutes.

Series II. Two dogs with Pavlov-type pouches, one dog with greater curvature, vagal denervated (Heidenhain) pouch and one dog with a pouch of the entire stomach, served as experimental animals in this series. Pilocarpine was employed as the gastric secretory stimulus. One test per animal per day was performed. On alternate days 100 cc. of 50 per cent olive oil emulsion was administered by stomach tube one hour prior to the injection of pilocarpine. Gastric juice was collected for 90 minutes after stimulation. The responses on the days on which olive oil was not administered served as control values.

Series III. A dog with esophagotomy, duodenal fistula, Heidenhain type pouch and a pouch of the remainder of the stomach with intact innervation was

employed in these experiments. This animal was prepared by transecting the pylorus of a dog which had been provided with a Heidenhain type pouch several months previously. A rubber retention catheter was sewn into the duodenal opening and the pylorus was brought out through a stab wound to serve as the stoma for the innervated pouch. Feeding was accomplished by way of the duodenal tube using the pabulum of Scott and Ivy (18). At a third operation an esophagotomy was performed to prevent contamination of gastric juice by saliva. On this animal simultaneous comparative observations could be made on a vagal innervated and a non-vagal innervated pouch. The double stimula-

TABLE 1

Averaged per cent change of second period values from first period values with standard error of per cent change

TYPE POUCH	STIMULUS	INHIBITORY PROCEDURE BEFORE SECOND PERIOD	NUMBER OF DOGS	NUMBER OF TRIALS	SECRETION DURING SECOND PERIOD AS PER CENT CHANGE FROM FIRST PERIOD				
					Volume	Acid conc.	Acid output	Pepsin conc.	Pepsin output
Denervated*	H**	None	7	12	+8±4	+8±4	+14±10	+136±36	+149±56
	H	Olive oil‡	6	18	−27±9	−22±8	−38±12	+117±29	+62±34
	P†	None	7	12	+2±8	+1±5	+5±8	+59±19	+65±26
	P	Olive oil	8	26	−29±7	−19±7	−37±10	+58±20	+7±17
	P	Enterog.§	7	12	−44±8	−28±7	−58±8	+134±27	+34±38
Innervated	H	None	2	10	−7±4	−5±3	−14±6	+110±22	+102±28
	H	Olive oil	2	12	−58±9	−52±10	−76±10	+84±29	−25±17
	P	None	2	10	−2±3	−3±3	−5±4	+48±20	+46±24
	P	Olive oil	2	12	−63±5	−42±11	−74±8	−55±7	−84±4
	P	Enterog.	2	10	−57±9	−57±10	−78±9	+109±19	+2±16

* Pouches of the entire stomach with vagi sectioned and subcutaneously transplanted pouches.

** Histamine dihydrochloride.

† Pilocarpine hydrochloride.

‡ 100 cc. of 50 per cent olive oil emulsion by stomach tube.

§ 50 mgm. enterogastrone intravenously.

tion plan of the experiments in Series I was followed except that olive oil was replaced by egg yolks and cream administered via the duodenal fistula.

RESULTS. *Stability of pepsin at pH values less than 5.* The gastric juice from the total gastric pouch of four dogs was studied by the technique outlined above in which the pH of the original juice was increased by the addition of appropriate acetate buffer solutions or weak HCl solutions of various known normalities. The results were similar to those obtained by Northrop (16) and, therefore, need not be presented. The peptic activity of the juice was not reduced until a pH more alkaline than 4.8 was reached.

Series I. The results are shown in table 1.

A. *Total Pouches with Vagi Sectioned and Subcutaneously Transplanted Pouches.* The results obtained using the subcutaneously transplanted pouches

showed no essential differences from the results on the pouches of the entire stomach; therefore the data are presented together.

(1) *Control series (no inhibitory procedure before second stimulation).* Volume, acid concentration, and acid output show a slight tendency to rise during the second period with histamine stimulation but not with pilocarpine stimulation. Pepsin concentration and pepsin output display a significant increase in the second period.

(2) *Inhibition by enterogastrone.* The injection of enterogastrone decreased the acid and volume output of the secretion. In regard to pepsin, the most uniform change was an increase in the concentration of pepsin even above the expected (control) rise during the second period. The effect on the total output of pepsin, however, was not uniform; in some animals there was no change, in others an increase or a decrease.

Inhibition by olive oil in the intestine. It was noted that pilocarpine-stimulated gastric secretion is not so uniformly or extensively inhibited by fat as it is by enterogastrone preparations in animals with a vagal denervated pouch. In general, the olive oil acted similarly to enterogastrone except that the pepsin concentration did not rise above the control level.

B. *Pavlov Pouches with Large Isthmus.* The control experiments with histamine revealed a slight tendency for volume, acid concentration, and acid output to fall during the second period in contrast to the tendency to rise of the vagally denervated pouches. Pepsin concentrations and output values in both pilocarpine and histamine control experiments were similar to those of the vagally denervated pouches, showing a distinct tendency to rise during the second period. Enterogastrone produced a decrease in volume and acid output, an increase in pepsin concentration above the control value, and a rise in pepsin output which was less than the increase which occurred in the control experiments. Olive oil produced a marked depression of volume and acid with both histamine and pilocarpine stimulation. Pepsin concentration is not greatly altered by olive oil acting against histamine stimulation but is greatly reduced in the case of pilocarpine stimulation. Olive oil decreases pepsin output slightly with histamine stimulation and markedly with pilocarpine stimulation.

Series II. The results are shown in table 2. Olive oil produced a decrease in volume in all cases and a decrease in acid in all but one dog. Pepsin concentration after olive oil was higher in the denervated pouches and lower in the innervated pouches. Pepsin output was unaltered in one denervated pouch, slightly reduced in the other, and greatly reduced in both innervated pouches.

Series III. Fat in the intestine inhibited the volume and acid secretion of the innervated pouch markedly and of the denervated pouch moderately. Pepsin concentration is not markedly altered by fat in the denervated pouch but is substantially depressed in the innervated pouch. Likewise pepsin output in the denervated pouch is not significantly altered by fat but is greatly inhibited in the innervated pouch.

Discussion. *Inactivation of pepsin at pH's below 5.* The report that pepsin is inactivated at pH's well in the acid range is based on experiments in which

adjustment of reaction of the medium was accomplished by the addition of dilute alkali. Such a procedure results in local exposure of a portion of the pepsin to an alkalinity capable of irreversibly denaturing it before thorough mixing can be accomplished. Our results show that as long as the gastric juice has a pH of less than 5 there is no reason to believe that some of the secreted pepsin has been inactivated. Furthermore, inasmuch as our results agree with those of Northrop (16), it is concluded that the susceptibility of the pepsin in gastric juice to inactivation by exposure to various hydrogen ion concentrations is essentially the same as that of chemically purified pepsin prepared from gastric mucosa.

Series I. The values for volume and acid tend, during the second period, to rise in the denervated pouches and fall in the innervated pouches. In general, however, these values are reasonably comparable for the two periods con-

TABLE 2

Averaged responses of vagal innervated and vagal denervated pouches to pilocarpine with and without olive oil

DOG	TYPE POUCH	INHIBITORY PROCEDURE	NUMBER OF TRIALS	VOLUME	FREE ACID CONC.	TOTAL ACID CONC.	TOTAL ACID OUTPUT	PEPSIN CONC.	PEPSIN OUTPUT
				cc.	*mgm./cc.*	*mgm./cc.*	*mgm.*	$U \times 10^4/cc.$	$U \times 10^4$
1	Entire stomach, vagi sectioned	None	5	31.0	2.4	3.3	115	53	1898
		Olive oil	5	25.0	0.9	1.9	53	80	1878
2	Heidenhain	None	5	11.3	2.3	8.4	32	27	323
		Olive oil	5	5.5	1.4	1.9	12	38	207
3	Pavlov	None	5	11.6	1.1	2.0	23	69	818
		Olive oil	5	7.7	0.7	1.3	10	13	102
4	Pavlov	None	5	20.0	1.3	2.1	43	75	1514
		Olive oil	5	14.8	2.5	3.2	51	21	348

sidering the tendency for variation in response in gastric pouch dogs. Pepsin concentration and output displays a significant increase in the second period in both innervated and denervated pouches. This tendency of pepsin concentration to rise on repeated stimulation with histamine has been reported by Lim and Ma (19) using hourly injections for 24 hours and by Bucher (20) using injections every ten minutes. However, both Bucher (17) and Rivers (21) found that when only 2 injections of histamine were given one hour apart the pepsin response in the two periods was almost identical. The reason for this increase in pepsin concentration and output with repeated stimulation is obscure.

It must be borne in mind, in interpreting the data in Series I as presented in Table 1, that variations in pepsin concentration and output following an inhibitory procedure must be compared with the variations from the first period which occur in the control series. Thus, for example, the increase in pepsin concentration with histamine stimulation in the denervated pouch following

olive oil was not notably different from the increase which occurred during the second period when no inhibitory procedure preceded the second stimulation. This, then, would represent no alteration in pepsin concentration due to olive oil. This applies particularly to the pepsin values where the variations from the first period were large in the control experiments. Whenever variations in pepsin secretion are ascribed to the inhibitory procedure in the following discussion, it is this variation from the control value and not the variation from zero which is intended.

On this basis the following observations are presented: *a.* As we have noted before, the depression by olive oil of acid and volume of secretion is more extensive in dogs with part of the stomach remaining in the alimentary circuit than in dogs with pouches of the entire stomach. This is probably due to a more prolonged and continous exposure of the upper intestinal mucosa to the

TABLE 3

Response of dog with a vagal innervated and a vagal denervated pouch to pilocarpine stimulation with and without fat in the duodenum

TRIAL NUMBER	PERIOD NUMBER	VOLUME		FREE ACID CONC.		TOTAL ACID CONC.		TOTAL ACID OUTPUT		PEPSIN CONC.		PEPSIN OUTPUT	
		I*	D**	I	D	I	D	I	D	I	D	I	D
				mgm./cc.	*mgm./cc.*	*mgm./cc.*	*mgm./cc.*	*mgm.*	*mgm.*	*U × 10⁴/cc.*	*U × 10⁴/cc.*		
1	1†	6.8	4.5	1.8	2.9	2.4	3.4	16	15	122	58	830	261
	2‡	2.9	4.0	0.1	2.0	0.6	2.3	2	9	54	61	157	264
2	1	8.7	5.0	2.0	3.0	2.8	3.6	24	18	158	63	1375	315
	2	2.6	4.0	0.4	1.9	0.9	2.3	2	9	52	70	135	280

* I, vagal innervated pouch.
** D, vagal denervated pouch.
† Control or first period.
‡ Second period, after instillation of fat into duodenum.

fat in the former animals. *b.* The most remarkable alteration in pepsin secretion is a pronounced depression of pepsin concentration and output when pilocarpine stimulation is inhibited by olive oil. It is to be noted that significant depression of pepsin concentration occurs in no other experimental procedure used in this study. Whether this depression of pepsin concentration is also to be attributed to the factor of prolonged action of the fat in the Pavlov dog (as discussed above) or to the integrity of the vagal nerves in these animals was investigated in the experiments of Series II and III. *c.* Endogenous and exogenous enterogastrone cause moderate inhibition of pepsin output when vagal influences are excluded. *d.* Parenteral administration of enterogastrone increases the concentration but decreases the total output of pepsin with pilocarpine stimulation in both vagal innervated and vagal denervated pouches.

Series II. In our experience, in unselected gastric pouch dogs, the variability in volume and acid secretion is greater when comparison is made between one

test performed on each of two days than when made between two tests on a single day. Three of the dogs were selected for this series on the basis of known uniformity of response to pilocarpine as regards volume and acid. Dog 4 displays the variability from day to day in acid response characteristic of unselected gastric pouch dogs. Although the number of trials are too small to permit statistical analysis, inspection of the individual values reveals that the increase in average concentration and output of acid in dog 4 is not significant because of the great variability. On the other hand, the values for pepsin concentration and output are quite homogeneous and therefore, differences between means are significant.

In series II, as in series I, marked depression of pepsin concentration was noted only in the vagal innervated pouches.

The Pavlov pouch preparation differs from the total pouch in two respects: 1, a portion of the stomach remains in the alimentary circuit; 2, the pouch receives vagal innervation. The Heidenhain pouch dog was used to investigate the possibility that the marked depression of pepsin concentration and output noted in the Pavlov pouches in Series I was due to the presence of a portion of the stomach in the alimentary circuit and not to its vagal innervation. The presence of a portion of the stomach in the alimentary circuit might conceivably be responsible for pepsin inhibition by *a*, providing a more prolonged and continuous exposure of the upper intestine to the fat, or by *b*, being the site of formation of a hormonal substance inhibiting pepsin secretion. Inasmuch as the results on the Heidenhain pouch were not greatly different from those on the total pouch animals, neither of these possibilities is supported, and the differences noted must be attributed to the presence of vagal influences in the Pavlov pouch animals.

Series III. In order to further define the rôles of nervous and humoral factors, an experimental animal was provided in which the action of fat on pepsin secretion could be observed simultaneously in two pouches which differed only in that one retained its vagal innervation. The factor of length and extent of exposure of the intestine to fat was the same for both pouches, likewise the stimulus to the two pouches was identical. The findings confirm those of the other series of experiments in that marked depression of pepsin concentration and output occur only in the pouch in which vagal influences are operative.

SUMMARY AND CONCLUSIONS

The inhibitory effect of enterogastrone on the gastric glands of the dog is characterized by the following alterations which are apparent when enterogastrone concentrates are administered parenterally and when endogenous enterogastrone production is elicited by the presence of fat in the intestine: Volume of secretion and acid concentration are moderately inhibited and, thus, acid output is markedly decreased. *Pepsin output* is moderately depressed by enterogastrone in the absence of vagal influences. These alterations occur with either histamine or pilocarpine stimulation.

Depression of pepsin *concentration* after fat administration occurs only in the vagal innervated pouch.

Vagal nervous influences are extensively concerned in the inhibition by fat of pepsin secretion.

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THE EFFECT OF EXTERNAL CONSTRICTION OF A BLOOD VESSEL ON BLOOD FLOW[1]

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Received for publication January 14, 1944

In many situations in which blood flow through a vessel is under consideration, it is frequently a matter of practical importance to estimate the reduction in blood flow which will result when the lumen of a blood vessel is partially obstructed or locally reduced by compression. As examples, a vessel lumen may be reduced from natural causes by external compression (e.g., extravascular muscular contraction, neoplastic masses), intrinsic pathologic changes (sclerotic plaques, scars), or artificially reduced by vessel clamps (Goldblatt clamps and other devices), flow measuring instruments (thermostromuhr, electromagnetic flow recorder), etc. Considering the diversified fields in which such information might be of practical value, little physiological work has been published which deals with the relationship of vessel bore to volume flow.

Interest in this problem was aroused during the course of recent experimental studies in this laboratory on the accuracy of the thermostromuhr method for measuring blood flow (1, 2). It was observed that the application of a loosely fitting thermostromuhr unit to an artery frequently caused an appreciable decrease in the flow through the artery as measured by a rotameter (3). While it did not seem likely at the time that the lumen was markedly reduced by application of the thermostromuhr, no vessel diameter or wall thickness measurements were made from which values of lumen area could later be computed. These observations suggested the possibility that a relatively small external constriction of an artery could, under physiological conditions, induce an appreciable reduction in flow through a vessel.

The study of vessel constriction and its flow limiting effects involves consideration of the various factors which determine the rate of blood flow through a given vessel segment. An expression of the interrelationship of these factors is given in Poiseuille's law which states that the rate of flow through small tubes is directly proportional to the 4th power of the radius of the lumen and to the pressure drop across the two ends and inversely proportional to the viscosity of the blood and the length of the tube. Thus, for any vessel segment which is locally constricted, the accompanying reduction in blood flow will become greater as 1, the lumen is decreased; 2, the length of the segment constricted is increased, and 3, the viscosity of the blood is increased. If, in a given case, these factors remain fixed, the rate of flow through the constriction will be governed entirely by the pressure difference across the two ends of the constricted segment. The factors which determine this pressure difference in an

[1] The expenses of this investigation were defrayed by a grant from the Commonwealth Fund.

intact artery are 1, the blood pressure on the upstream side of the constriction; 2, the resistance to flow through the constricted segment, and 3, the peripheral resistance of the arteries and vascular bed distal to the constriction.

Before proceeding to intact vessels in the animal, experimental determinations of flow through various constrictions were first made in an artificial system in which the above factors could be more easily varied or controlled. The apparatus used consisted of a reservoir tank (30 cm. in diameter) to the bottom of which was connected a rubber hose of large diameter (I.D. = 2 cm.). A metal tube, 10 mm. in length with a cylindrical lumen of 0.5, 1.0, 1.5, 2.0, 2.5 or 3.0 mm., was inserted into an adapter fitted to the distal end of the hose. A peripheral resistance (PR) was introduced distal to the constricting tube and separated from it by a 10 cm. length of large diameter hose (20 mm. I.D.). For the PR mechanism, one of a similar set of metal tubes 20 mm. long (I.D. ranging from 0.5 to 3.0 mm.) was used. Acacia solution with a viscosity approximating that of blood (4) (specific viscosity 4.0–4.5) was allowed to flow by gravity at a constant hydrostatic pressure, the outflow from the PR tube emerged into a vessel of the same fluid and the overflow was measured with a graduate and stop watch.

In figure 1 are plotted percentage reductions in lumen area of the constricting tube versus percentage decrease in flow when different peripheral resistances were imposed distal to the constricting tube. The internal diameters of the PR tubes are indicated beside the respective curves. In the high PR range (small bore PR tubes), the constricting tubes limit flow very little until the percentage decrease in lumen area is very high. When the PR is low (large bore PR tubes), the constricting tubes are much more effective in limiting flow.

From these experimental data it is evident that the PR mechanism at the end of the flow circuit (distal constriction) plays an extremely important rôle in determining the extent of flow limitation caused by the constricting tube (proximal constriction). One would anticipate quite similar variations in flow reduction in an *in vivo* preparation where the PR of a given vascular bed may undergo considerable change through vasodilatation or constriction. Accordingly, experiments were extended to anesthetized dogs in which were studied the effects of localized arterial constriction upon the flow of blood within an artery.

For this study, the common carotid artery was chosen because of its accessibility and length. Dogs, weighing 18–20 kgm., were anesthetized[2], given anticoagulants[3], and a large rotameter (3) with large bore cannulae inserted between the cut ends of one common carotid artery. Mean blood pressure was recorded by a mercury manometer connected to the rotameter. For a number of determinations the peripheral resistance of the bed was temporarily lowered, either by nitroglycerine injection (0.00016 gram) into the same artery (via a side tube on the rotameter), or by occluding the artery for 2–3 minutes prior to a determination, thereby producing in the latter case an ischemic dilatation within the corresponding bed. All of the experiments were of short duration and the condition of the dogs was excellent throughout.

[2] Sodium pentobarbital, 20 mgm. per kilo.

[3] Heparin, 100 U. per kilo and pontamine fast pink, 150 mgm. per kilo.

For external constriction of the artery, bivalved Lucite blocks, 10 mm. long, with centrally placed cylindrical holes of graduated diameters (0.5–5.0 mm.) were applied to the vessel peripheral to the rotameter, and the mean flow noted before, and immediately after placement around the artery. The external diameter of the artery (without constricting block) was determined frequently during each experiment by encircling the vessel three times with a fine silk thread, tying the knot snug to the artery, cutting the thread and measuring, while still wet, its length (circumference × 3) from which the external diameter was computed. At

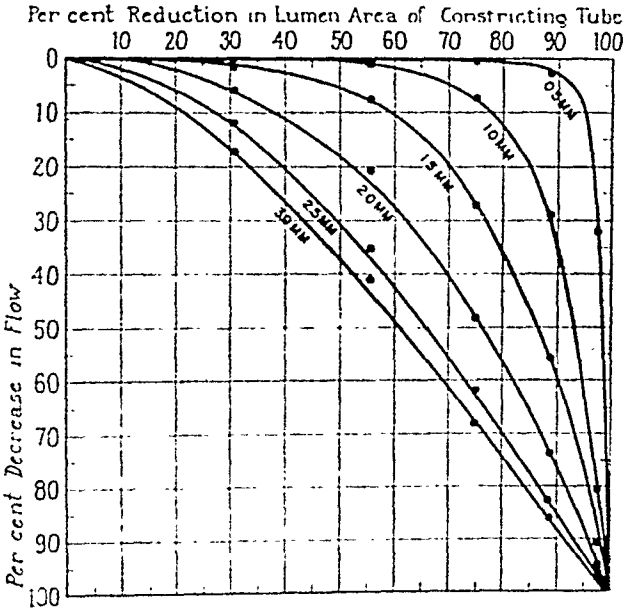

Fig. 1

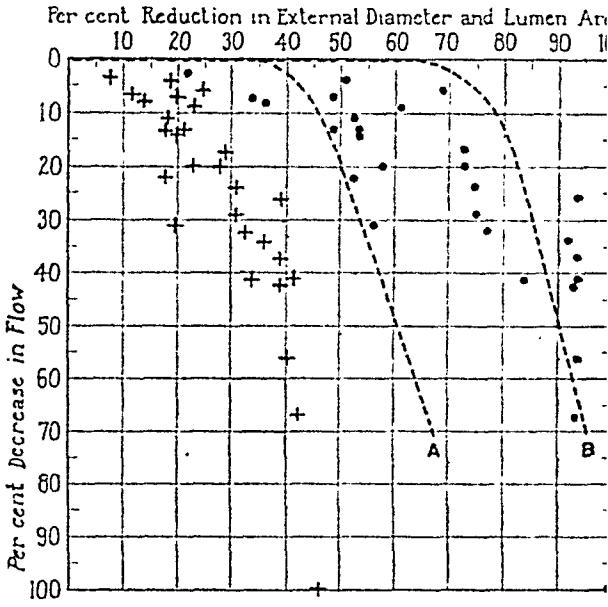

Fig. 2

Fig. 1. Graph showing the variations in flow reduction caused by fixed constricting tubes when different peripheral resistances were imposed distal to the constricting tube. Fixed constricting tube diameters—2.5, 2.0, 1.5, 1.0, 0.5 mm.; reference constricting tube diameter—3 mm. Opposite each curve is indicated the lumen diameter of the corresponding peripheral resistance tube; acacia solution (specific viscosity 4.0–4.5) used as fluid medium. Further description in text.

Fig. 2. Percentage reductions in blood flow plotted against percentage decrease in external diameter (+) and lumen area (.), of intact blood vessels in anesthetized dogs. The two broken line curves represent the relationship of external diameter (A) and lumen area (B) to percentage reduction in flow, as reported by Mann and co-workers. (See text.)

the end of the experiment and without disturbing the artery length, a small glass rod of measured diameter was inserted into the arterial lumen through a slit made in the side of the vessel and the circumference × 3 again taken. From the latter data, the cross section area of the *arterial wall* was determined. The lumen area of the vessel within the constricting block (total area minus wall area) was computed in the same way except that the external diameter and total cross section area of the vessel automatically becomes the same as the known internal diameter and lumen area of the constricting block. This method for determining the size of the lumen is based upon the assumption that the vessel is not appreciably extruded from the ends of the constricting block during application of the latter.

Such would diminish the cross section area of the arterial wall and thereby increase relatively the lumen area. However, in actual practice, direct observation of the vessel at the point of emergence from the constricting block revealed no visible extrusion or elongation during application of the constricting blocks.[4]

Of four similar animal experiments, the combined results of two typical ones are presented in figure 2 in which are plotted the percentage reductions in flow versus percentage reductions in external diameter (+) and lumen area (●) of the artery. Each point indicates the percentage change from the control to the mechanically constricted state of the artery. In these experiments, the blood flow ranged from 140 to 828 cc. per minute under different conditions and mean blood pressure remained constant (within 2–3 mm. Hg) during the actual determinations.

In contrast to the orderly sequence of changes in flow observed with the gravity system (fig. 1), the same relationships recorded *in vivo* with successive constrictions were decidedly irregular. Scattering of the points was observed even though the PR of the bed was not intentionally altered. When nitroglycerine was given or hyperemia induced, the points were even more irregular with a proportionately greater decrease in flow in relation to the percentage constriction.

Discussion. The relationship of flow, peripheral resistance, and applied pressure to the added resistance of luminal constriction can be most simply illustrated by reference to the somewhat analogous flow of current in an electrical circuit. According to Ohm's law, the flow of current is determined by $\frac{\text{voltage (pressure)}}{\text{Resistance}}$. Substituting hemodynamic equivalents, the rate of flow of blood through a given artery will be proportional to the applied pressure divided by the total resistance peripheral to that point in the vessel. As a working example, let us say 2 cc./sec. will flow through an artery when the applied pressure is 120 mm. Hg and the peripheral resistance is 60 arbitrary units—then $\frac{120}{60} = 2$. Assuming that a given localized constriction of the artery will add 20 units of resistance, then the flow will decrease by *25 per cent* to 1.5 cc./min. $\left(\frac{120}{60 + 20} = 1.5\right)$. If at another time, the peripheral bed is made to dilate so that its PR becomes 40 units, then the control flow will be 3 cc./min. $\left(\frac{120}{40} = 3\right)$. The addition of the same local

[4] A direct test of the method was made by observing the changes in arterial bore by means of a low power microscope fitted with an ocular micrometer. A recently isolated artery segment, tied to a cannula with a plane glass window at the end, was held in place with its long axis parellel to that of the microscope and inflated with 100 mm. Hg pressure. By transilluminating the arterial wall, the bore of the vessel could be visualized and measured at a focal plane 1.5 cm. from the end of the artery segment. Constricting blocks were applied at this level and the changes in bore observed directly. Close agreement between the values obtained by direct observation and those which were computed from circumference and wall area measurements permitted the use of the indirect method in the experiments to be presented here.

constriction (20 units of resistance) will now decrease the flow by *33 per cent* to 2 cc./min. $\left(\frac{120}{40 + 20} = 2\right)$. In other words, the effectiveness of a given local constriction in reducing the flow will vary under different conditions of the peripheral bed and the flow reduction may be large or small depending upon whether the resistance offered by the constriction is large or small in proportion to the *total resistance* (i.e., constriction resistance + peripheral resistance).

It is evident from the wide scattering of points in figure 2 that there exists no fixed relationship between percentage reduction in flow and percentage reduction in the luminal or external dimensions of a vessel *in vivo*. One would not expect that the orderly sequence of changes resulting from the introduction of a single variable in an artificial system (as in fig. 1) would be reproduced in the living animal since, for the same vessel, the blood pressure, peripheral resistance and

TABLE 1

Relationship of changes produced in the dimensions of the same vessel by external constriction under different assumed vasomotor conditions

OUTSIDE DIAMETER			INSIDE DIAMETER			LUMEN AREA		
Value	Incremental % decrease	Cumulative % decrease	Value	Incremental % decrease	Cumulative % decrease	Value	Incremental % decrease	Cumulative % decrease
mm.			*mm.*			*sq.mm.*		
5.0	Initial		4.35	Initial		14.9	Initial	
4.5	*10*	10	3.77	*13.3*	13	11.1	*25.0*	25
4.05	*10*	19	3.22	*14.6*	26	8.14	*27.0*	45
3.64	*10*	27	2.69	*16.5*	38	5.67	*30.3*	62
3.28	*10*	34	2.17	*19.4*	50	3.69	*35.0*	75
2.95	*10*	41	1.63	*24.9*	63	2.08	*43.6*	86
2.66	*10*	47	1.00	*38.6*	77	0.79	*62.0*	95

rate of flow will undergo physiological variations from time to time. These factors will also vary from animal to animal and among different vessels in the same animal.

Of equal and perhaps greater importance is the fact that the wall area, external diameter, and lumen diameter may be considerably different from vessel to vessel, and, in the case of the latter two factors, even for the same vessel under different vasomotor conditions. The relationship of changes in external diameter to changes in lumen area (and flow) may therefore be equally variable. The magnitude of variation in this relationship is illustrated in table 1 in which are presented the calculated percentage and absolute changes in the dimensions of a single vessel which will result from a 10 per cent reduction in external diameter with different pre-existing vasomotor states. These data illustrate (and mathematical considerations dictate) that as the external vessel diameter progressively diminishes (as in the case of active vasoconstriction) the same percentage (10 per cent) decrease in the *existing* external diameter will cause an *increasingly greater percentage decrease in the existing lumen area* (cf. italicized figures, table 1).

An additional and equally unpredictable factor which may alter the ultimate influence of a localized constriction upon blood flow is the response of the peripheral bed. When the flow to the bed is reduced by the constriction, the peripheral vessels may dilate as a result of the associated ischemia and the flow may tend to increase, the combined resultant of which will be a new equilibrium. For this reason it is probable that the amounts by which blood flow was reduced in the foregoing animal experiments are less than those which would be recorded if it were possible to prevent the partial compensation (by dilatation) for the ischemia of the bed peripheral to the constriction.

The foregoing considerations reveal that it is impossible to predict within rather wide limits the amount of flow reduction which will result when an intact vessel is constricted by external means. However, from a physiological standpoint, a reduction of the lumen of a vessel may be of little functional importance to the vascular bed supplied by that vessel when the rate of blood flow is already low, but on the other hand, the same constriction may seriously limit the blood supply to the same bed just at the time when the requirements of the latter are greatest and flow would otherwise be much greater.

Many of the findings, interpretations and conclusions presented here differ from those reported by Mann, Herrick, Essex and Baldes (5). These investigators studied the effects of vessel constriction in an artificial system and *in vivo*, and concluded that blood flow through a vessel is not reduced by a significant amount unless the cross section area of the lumen is reduced by 50–70 per cent. Unfortunately, certain differences in the procedures and methods of study make it impossible to compare much of their data with that presented here. As examples, 1, in their experiments with an artificial circulation system, a peripheral resistance was maintained by capillary tubes or a "satisfactory mechanism." The use of a single, arbitrarily chosen peripheral resistance will not reveal the wide range and variability of the flow reductions which can be caused by a given constriction. (See family of curves, fig. 1.) 2. In their communication, a progressive external constriction of a vessel in the living animal is reported to have caused the lumen to decrease in size as illustrated in the scale drawings of figure 3A. With the use of constricting units 10 mm. long, it is difficult to conceive of a mechanism by which *a*, the wall thickness would remain the same throughout the various stages of constriction, and *b*, the external diameter of their vessel could be reduced 57 per cent and 68 per cent without obliteration of the lumen and cessation of flow. In figure 3B are shown the successive changes in the dimensions of the same vessel which have been calculated mathematically in accord with geometric principles. 3. In the same experiments, the flow reductions attributed to the application of constricting units cannot be considered maximal since the thermostromuhr itself (used in the intact vessel experiments to measure the flow in the artery) may have already constricted the lumen to such an extent[5] that blood flow is initially reduced by a significant amount. Its effect would be that

[5] "... successful measurement of blood flow necessitates the application of [thermostromuhr] units which have internal diameters somewhat less than the external diameters of the blood vessels under investigation."—Mann, Herrick, Essex and Baldes (5).

of introducing an additional resistance which, according to the findings shown in figure 1 diminishes the flow-reducing effect of the experimental constriction. Further, the thermostromuhr cannot be regarded as an adequate instrument for the measurement of blood flow (1, 2, 6, 7).

Irrespective of the validity of the methods used, the conclusions of Mann and co-workers can apply only to the special cases from which they were derived and certainly not to all vessels under a variety of conditions. (See curves A and B versus plotted points in fig. 2.)

The most important conclusions to be drawn from the present study are that: the reduction in flow caused by external constriction of an intact vessel 1, cannot

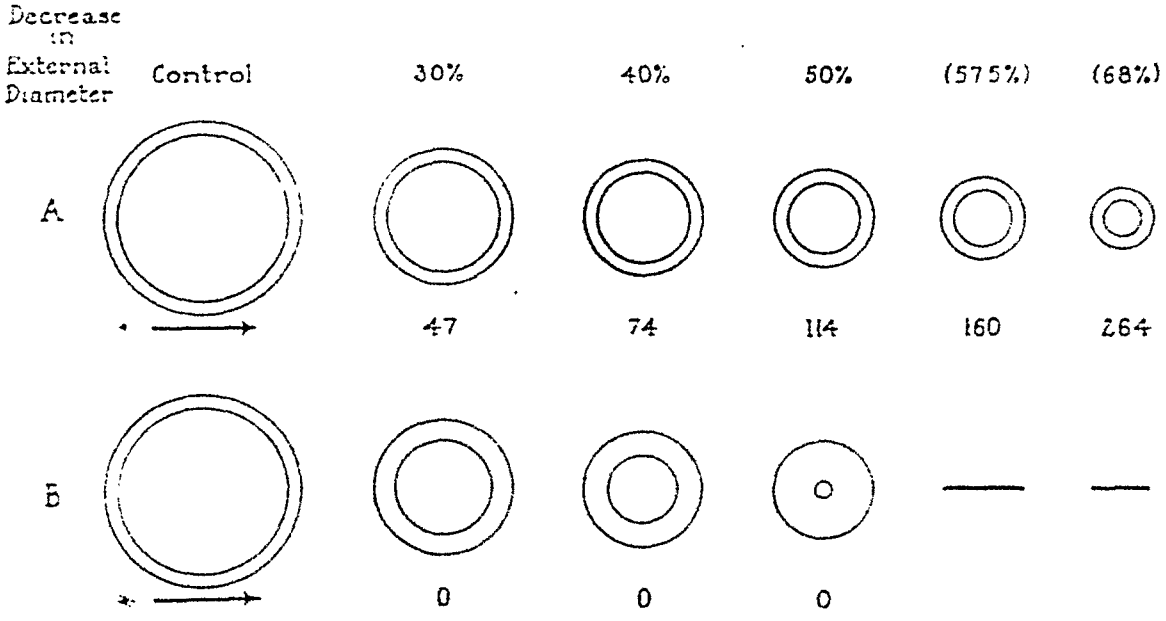

Fig. 3. Part A—Scale drawings of the changes in the dimensions of an artery produced by external constriction, constructed from the data of Mann. Part B—Theoretically expected changes in the dimensions of the same vessel computed on the basis that there is no extrusion of the vessel and consequently the arterial wall becomes thicker as the vessel is constricted. (* ←—Percent elongation or extrusion of the vessel which would be required to maintain wall and luminal dimensions indicated. Control dimensions: External diameter 5 mm., internal diameter 4.35 mm. See text for further description.

be predicted; 2. may vary through wide limits under different conditions; 3. may or may not cause significant metabolic embarrassment immediately to the organ it supplies, and 4. if not immediate, the same constriction may still limit flow in greater proportion at a later time when requirements of the organ may be increased and flow through the constricted vessel would otherwise be greatly augmented. 5. It is hazardous to assume that a very small external constriction will limit flow very little since vessels in (unsuspected or unidentified) vasomotor constriction may possess lumina so small that very slight additional mechanical constriction could cause an almost, if not complete, occlusion. 6. An examination of a diseased vessel post mortem is of questionable value in estimating the degree of flow reduction during life since with a dead vessel, the vasomotor state,

vessel dimensions, degree of distention (by intravascular pressure) and vessel constituents (edema, dehydration, etc.) all are usually far removed from their physiological ranges which existed during life.

SUMMARY

The effect of an external constriction of a blood vessel in limiting blood flow has been considered with respect to the relationships of 1, vessel bore to volume flow, and 2, change in external to change in internal dimensions of the vessel. Experiments with an artificial system and in animals have led to the conclusions that:

The effect of a localized reduction in lumen area is primarily that of increasing the fluid friction (viscosity effect) at the site of the constriction, which results in an added "peripheral resistance" to the flow of blood and the rate of flow is thereby reduced.

The extent of flow reduction will vary in direct relation to the axial length of the constricted area, the velocity of flow and the viscosity of the blood, and in inverse relation to the peripheral resistance of the bed and the lumen area of the vessel constriction. Since, with an intact blood vessel, it is impossible to determine all or even most of the above factors, an estimation of the flow reduction caused by a given constriction will be only as accurate as the estimated values placed upon the determining factors. Without the observer's knowledge, marked changes in the determining factors may occur, thereby making it impossible to predict within rather wide limits either the immediate or subsequent effects of a known constriction.

The findings presented here reveal no justification for the contention that a rather marked degree of external constriction is required to produce a significant reduction in flow through a vessel.

In comparison with other external constricting devices, the thermostromuhr cannot be regarded as having any less variable or unpredictable effect in limiting the rate of flow through the vessel to which it is applied.

The authors wish to express their appreciation to Dr. T. G. Bidder for assistance in some of the experiments.

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ERRATUM

On page 19 of the current volume (Vol. 141) figures 1 and 2 for the paper "The Effect of Carbon Monoxide on the Oxyhemoglobin Dissociation Curve" by F. J. W. Roughton and R. C. Darling were inadequately reproduced. Below these figures are again reproduced with satisfactory legibility.

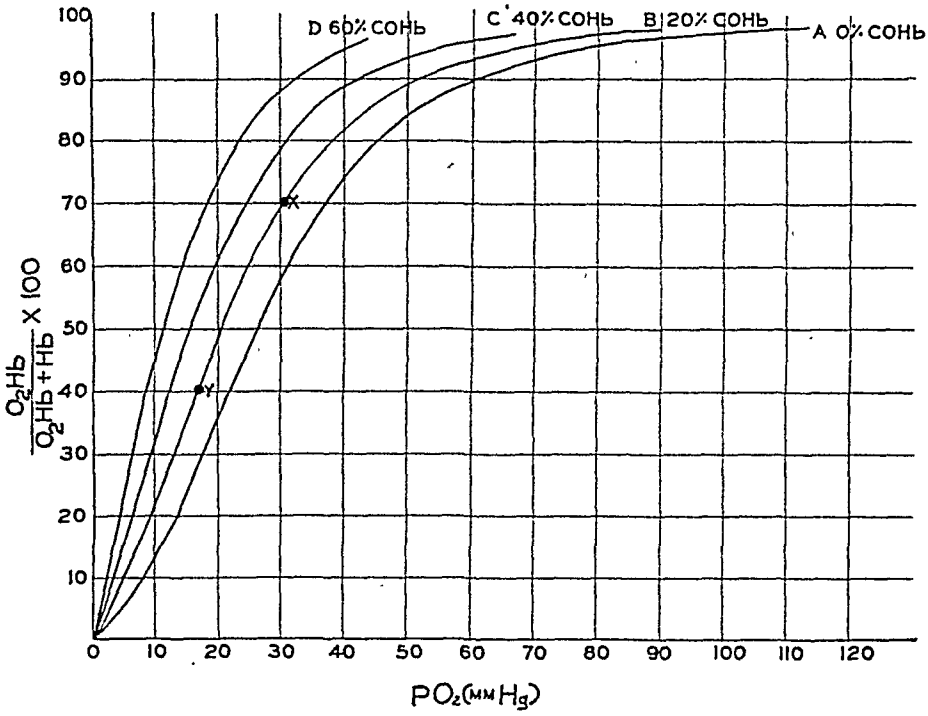

Fig. 1. Oxyhemoglobin dissociation curves of human blood containing varying amounts of carboxyhemoglobin, calculated from the observed O_2-dissociation curve of CO free blood.

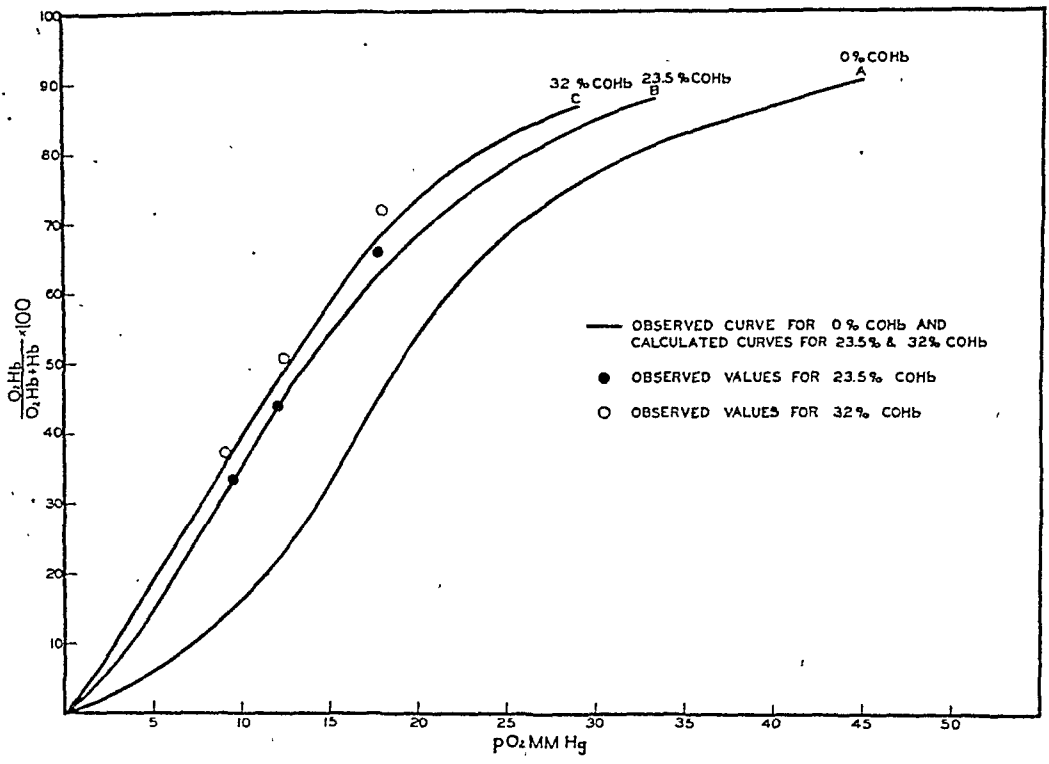

Fig. 2. Observed and calculated oxyhemoglobin dissociation curves of buffered human hemoglobin solutions containing varying amounts of carboxyhemoglobin.

THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 141 MAY 1, 1944 No. 3

INFLUENCE OF GLUCOSE ON THE GASPING PATTERN OF YOUNG ANIMALS SUBJECTED TO ACUTE ANOXIA

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Received for publication December 24, 1943

The prolonged survival of young animals subjected to acute anoxia has received the attention of a number of workers (1, 2, 3, 4). The writer has pointed out that the respiratory center of young animals itself is much more resistant to anoxia than is that of the adult and that until approximately six weeks of age survival of the primitive respiratory mechanism (gasping) is inversely proportional to age (5). Following decapitation, gasping of the isolated head of the rat continues for 30 minutes or more in the newborn but for only 10 or 15 seconds in the adult. Similar observations have been made on rabbits and dogs (6). This increased tolerance of young animals to anoxia is apparently due to several factors: *a*, low metabolic rate of the central nervous system; *b*, poikilothermia, and *c*, an anaerobic source of energy.

Himwich and associates (7) have studied the source of this anaerboic energy and found that insulin reduces and glucose increases the survival of young animals placed in pure nitrogen. Such findings emphasize the importance of carbohydrate stores in the tolerance of these animals to anoxia. The present studies were undertaken to determine whether carbohydrates influence the gasping pattern of the isolated unperfused head in which only the stored nutrients of the nervous tissue are immediately available. The respiratory center of such a preparation, unlike that of the whole animal with circulation intact, cannot be influenced secondarily by changes in other parts of the body.

METHODS. Employing a technique previously described in determining the influence of various chemical agents on the survival of the primitive respiratory mechanism (8), young rats, 12 to 15 days of age and weighing 18 to 26 grams, were used as test animals. Since the survival time, as well as the total number of gasps, is altered by litter or family characteristics and by the nutritive condition and degree of hydration of the animal (9), the experiments were carried out on litter mates subjected to identical cage conditions.

The gasping pattern for untreated animals of this age group consists of 25 to 33 mandibular movements lasting approximately four and one-half minutes and is composed of an initial or aerobic series having a relatively constant number

of gasps, averaging about 8, and a second or anaerobic series having a somewhat variable number (17–26) averaging about 21. In preliminary experiments on 35 litters comprising 245 animals, the standard deviation of the mean number of gasps for untreated litter mates was found to be slightly less than 2; the average variation for survival was 8.4 per cent and that for number of gasps of the second series was 6.1 per cent.

One hundred forty-four animals from 18 selected litters were removed from their respective mothers 12 to 15 hours prior to the experiment and members of each litter were divided by random sampling into 4 general groups, three of which were subjected to procedures intended to alter the supply of immediately available carbohydrates. One group, composed of two to four animals from each of the 18 litters, was injected with 4 or 8 units of insulin[1]; another group, composed of a similar number of animals from each litter, was given 0.4 cc. of 10 per cent glucose; a third group, composed of one animal from each litter, was given 8 units of insulin, two hours after which 0.4 cc. of 10 per cent glucose was also injected. Controls were given 0.4 cc. of 0.9 per cent NaCl. Five additional litters, comprising 43 animals, received slightly different treatment; the dosage for insulin and glucose was increased and the interval between injection and decapitation was more variable. All injections were made subcutaneously.

After intervals ranging from 30 minutes to 5 hours, the head was quickly isolated by guillotine methods and the ensuing gaps were recorded mechanically by means of a light heart lever attached to the mandible. Due to the influence of temperature on frequency and duration of gasping (9), the experiments were carried out under a constant room temperature of 31°C.

RESULTS. Results for the 18 litters receiving uniform treatment have been summarized in table 1. It is noted that although the extreme values of the treated groups show marked variations, the average values are definitely altered by the various procedures.

Controls. Following the onset of anoxia, the isolated head of control animals gasped a total of 29.8 times over a period averaging 319 seconds. The respiratory pattern for this group consisted of 1, an initial series of 8.5 (7–12) gasps lasting 18.6 (16–22) seconds; 2, a pause (interseries interval) lasting 38.7 (34–45) seconds, and 3, a second series of 21.3 (18–25) gasps lasting 262 (210–315) seconds.

Insulin-treated. Survival of respiratory activity in the insulin-treated animals was variable but definitely shorter than that of the controls and proportional to the severity of insulin action. Although the first series, like the second, was ultimately reduced in number and duration, the number of gasps was actually increased during moderately severe insulin reactions; in some instances this amounted to 40 per cent or more. The maximum average increase (+22.4 per cent) occurred 3 hours after injection of 8 units; at this time the duration of the series was slightly reduced (−10.6 per cent). Frequency was therefore

[1] The insulin dosage is massive but it is well established that infant animals are highly resistant to the hormone; convulsions are seldom, if ever, observed in young rats of the age group employed.

increased. Five hours after 8 units the number of gasps was reduced (−13 per cent) and the duration further shortened (−28 per cent).

TABLE 1

Influence of carbohydrate stores on the gasping pattern of the isolated head of 12–15 day old rats

EXPERIMENTAL GROUP AND PROCEDURE	NUMBER ANIMALS OBSERVED	NUMBER GASPS IN SERIES I		DURATION SERIES I		DURATION INTERSERIES INTERVAL		NUMBER GASPS IN SERIES II		DURATION SERIES II	
		Average and extremes	Change	Average and extremes	Change	Average and extremes	Change	Average and extremes	Change	Average and extremes	Change
		sec.	%	*sec.*	%	*sec.*	%	*sec.*	%	*sec.*	%
Controls, 1 hour after 0.4 cc. 0.9% NaCl	18	8.5 7–12		18.6 16–22		38.7 34–45		21.3 18–25		262 210–315	
Glucose, ½ hour after 0.4 cc. 10% glucose	11	8.8 7–13	+3.5	19.7 16–24	+5.6	39.8 34–46	+2.8	24.6 19–32	+15.5	293 212–347	+11.8
Glucose, 1 hour after 0.4 cc. 10% glucose	18	8.7 7–12	+2.4	19.3 15–24	+3.5	40.6 35–46	+4.9	28.1 19–32	+31.6	341 240–418	+30.2
Glucose, 2 hours after 0.4 cc. 10% glucose	14	8.9 7–13	+4.7	18.8 16–23	+1.1	41.2 35–46	+6.5	27.5 19–35	+29.1	332 252–403	+26.8
Glucose, 3 hours after 0.4 cc. 10% glucose	12	8.9 7–13	+4.7	20.3 17–24	+8.6	39.6 34–46	+2.3	25.2 18–36	+18.3	295 210–380	+12.6
Insulin, ½ hour after 4 units	10	8.7 6–13	+1.2	19.6 16–24	+5.1	35.3 30–41	−8.8	18.2 12–26	−14.5	252 105–345	−3.8
Insulin, 3 hours after 4 units	12	9.7 7–15	+14.1	17.9 13–22	−9.1	28.7 22–37	−25.8	12.2 6–20	−42.6	90 23–180	−65.5
Insulin, 3 hours after 8 units	18	10.4 6–16	+22.4	16.8 12–22	−10.6	22.6 16–32	−41.5	4.4 3–12	−79.2	21 11–62	−92.0
Insulin, 5 hours after 8 units	13	7.4 4–10	−12.9	14.2 9–19	−27.8	20.1 15–30	−48.0	2.4 0–7	−88.6	15 0–45	−94.5
Insulin + glucose 3 hours after 8 units 1 hour after 0.4 cc. 10% glucose	18	8.8 6–13	+3.5	19.3 14–24	−2.1	39.8 31–45	+2.8	19.4 12–32	−8.9	278 189–392	+4.2

Another striking change produced by insulin involved the interseries interval, there being an inverse relationship between the interval and the severity of insulin action. Five hours after 8 units it was reduced by 48 per cent.

The second series was also greatly altered by insulin. Animals receiving 4 units and tested after 30 minutes showed only a slight decrease (−14.5 per cent) in number of gasps and no significant change in duration; animals tested after 3 hours showed a reduction of 43 per cent for number and 65 per cent for duration. Similar changes were occasionally found for larger doses and shorter post-insulin periods. Marked changes in number and duration were found 5 hours after 8 units, when values of −89 per cent and −95 per cent, respectively, were obtained. Under conditions of severe insulin reaction, most animals gasped only two or three times; terminal respiratory movements were frequently apneustic, inspiration being maintained for 10 to 15 seconds or even longer.

Glucose-treated. The effects of subcutaneous injection of glucose were equally striking. Although neither the first series nor the interseries interval was markedly affected, the duration and number of gasps of the second series were definitely increased. The maximal effect of glucose in this regard was observed 1 hour after injection, when values approximating +30 per cent were found for number of gasps and duration. The amplitude or vigor of individual gasps of the second series was increased in many instances. Frequency, however, was usually decreased during the middle third of this series, when the rhythm also was usually irregular. In some instances the number of gasps was increased without a concomitant increase in duration and frequency was thus increased. Double spiked or diphasic gasps usually appeared or became more frequent during the middle third of the series.

Glucose administered to animals injected 2 hours previously with 8 units of insulin restored respiratory activity to normal or nearly normal values within one hour. This recovery in activity was slightly more noticeable for duration of gasping (+4 per cent) than for number of gasps (−9 per cent). In some instances the values were increased beyond those of the control; this increase, however, was no greater than that observed for glucose alone.

Discussion. It is obvious that immediately available carbohydrates greatly influence the character and duration of the respiratory pattern of young animals subjected to acute anoxia. That the respiratory center, like other parts of the nervous system, makes its chief demand for prolonged activity on a carbohydrate fuel is also apparent. The results support the contention that the marked tolerance of young animals to anoxia is due primarily to an anaerobic source of energy.

The demonstration by Himwich and co-workers (3) of the survival of infant rats given sodium cyanide, which inhibits the cytochrome oxidase system, would seem conclusive evidence that anaerobic energy is available to young animals. The writer (9) has found that in proper dosage cyanide completely suppresses the first series of gasps without altering the second, and that acetoacetic acid inhibits the second series without altering the first. The latter tissue poison influences anaerobic impulse formation to such a degree that the second series may be completely blocked, total survival thereby being reduced to a short aerobic series of 6 to 9 gasps lasting only 15 to 20 seconds. In suppressing the anaerobic series, acetoacetic acid gives a gasping pattern resembling that of the

untreated adult or the young animal subjected to excessive insulin. Such results suggest the anaerobic conversion of carbohydrates to lactic acid as the source of energy for the prolonged respiratory activity of the isolated head of the young animal.

Holmes and Holmes (10) found that the concentration of cerebral lactic acid developing under anaerobic conditions is proportional to the level of blood sugar and that the latter is increased as a result of breakdown of brain glycogen. That insulin administered to normal cats decreases the level of both glycogen and free sugar in the brain was shown by Kerr, Hampel and Ghantus (11). In lowering the immediately available carbohydrate stores of the central nervous system, insulin diminishes access to the respiratory center of its most important substrate for aerobic and anaerobic energy.

Although the duration of gasping of the first series tends to decrease under the influence of insulin, the number of gasps may actually increase during moderately severe insulin responses; hence, it appears that a small amount of fuel, incapable of sustaining an anaerobic series, is capable of supporting an aerobic series having a normal or augmented number of gasps.

It is not easy to explain the increase in frequency of gasps of the first and second series during mild or moderately severe insulin reactions. It might be pointed out, however, that Gellhorn and Kessler (12) have reported increased brain potentials during insulin hypoglycemia in adult rats and cats. Nor is it clear why the interseries interval is shortened by insulin. Since the hormone diminishes the substrate involved in the release of anaerobic energy, the opposite effects might be expected.

In those experiments in which the carbohydrate stores of the brain were raised by injection of glucose, the increase in number and duration of gasps of the second series can be attributed to augmentation of this anaerobic source of energy. The marked increase in the formation of double gasps cannot, however, be adequately explained, nor can the decreased frequency and rhythmicity of the second series. These changes probably represent an imbalance between the various substrates and enzymes implicated in the release of energy through glycolysis, which is known to proceed over an intricate pathway involving five or more stages. High concentrations of glucose in the reticular gray matter of the medulla may alter impulse formation by disturbing the enzyme-substrate relationship of one or more stages, and in this way delay or otherwise modify the development of the unstable chemical-nervous state necessary for "toppling" (13).

SUMMARY

Striking changes in the character and duration of the gasping pattern of young animals subjected to acute anoxia are produced by procedures which alter the stores of immediately available carbohydrates. Administration of insulin several hours prior to isolation of the head results in a slight to moderate reduction in the duration of the aerobic gasps, a marked reduction in the duration of the interval between the first (aerobic) and second (anaerobic) series of gasps,

and a profound reduction in the duration and number of gasps of the latter series. The frequency of gasps of both series is increased after moderate amounts of insulin. Large doses of insulin may almost completely suppress the anaerobic series without greatly altering the aerobic. In the more responsive animals, the terminal gasps of the second series are apneustic, inspiration being maintained for 10 to 15 seconds.

Injection of glucose produces gasping patterns which are practically the reverse of those found for insulin. Survival of respiratory activity is increased by 30 per cent, the number and duration of the gasping movements being maximal 1 hour after administration. Glucose tends to diminish the frequency and the rhythmicity of gasping in the middle third of the second series, and to produce double gasps. Given to animals under the influence of large doses of insulin, glucose restores the various phases of the gasping pattern to normal or nearly normal values within 60 minutes.

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EFFECTS OF FASTING ON THE RESPIRATORY METABOLISM OF NORMAL AND HYPOPHYSECTOMIZED YOUNG PIGEONS

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Received for publication January 17, 1944

Though the gaseous metabolism of pigeons has been frequently measured, particularly in connection with studies in avitaminosis and pharmacology, practically nothing is known concerning the metabolism of pigeons during prolonged fasting. Current studies of this laboratory are concerned with effects of hypophyseal hormones on heat production and on carbohydrate and fat metabolism in normal and hypophysectomized young pigeons. Pigeons deprived of either the anterior pituitary, adrenals or pancreas voluntarily restrict their food-intake to about one-fourth of the normal (1, 2). Information concerning the nature of the tissue changes and gaseous interchanges in these animals while completely fasting is therefore especially desirable. Again, both the true value and the interpretation of the respiratory quotient in fasting birds are unsettled questions on which the present tests were expected to provide further information.

Rubner (3) reported that pigeons given no water die of thirst in 4 to 5 days, whereas those allowed water and no food live 12 days. Our own tests indicate somewhat longer survival. Four of 5 adult pigeons, hypophysectomized two weeks before the test, survived a fast of 20 days; one died on the eighteenth day. In another test (2) which preceded the present study all of 20 normal young of the breed used here, and all of 15 young which had been completely hypophysectomized on their first fasting day, survived a 10-day fast. In view of these results it seemed possible to subject these two types of birds to a fast of 10 days without encountering moribund animals at the termination of the test.

MATERIAL AND METHODS. White Carneau pigeons at 7 to 10 weeks after hatching, free from body lice and previously fed on a mixed grain diet, were used. Most birds were well protected against cold; but among two groups of operated birds tested at the end of August there were six deaths after 5 to 9 days of fasting; these deaths probably resulted from exposure to cold at night. One additional operated bird died in April on the eighth day of the fast. All birds were permitted to live in groups in rather large well-lighted cages; tap water (and sand) was constantly available. Groups of 4 or 8 normals, and other groups of 4 or 8 hypophysectomized birds, were measured simultaneously (or with one day of over-lap) during a 10-day period. Composite groups of this same type, however, were measured at different seasons of the year. Again, some birds were operated before and some after the initiation of their fast in order that possible effects of narcosis (nembutal) incident to hypophysectomy might be better observed or controlled. The completeness of all operations (anterior lobes only were removed) was tested by section and microscopic examination of any suspicious tissue found in the sella at autopsy.

At the termination of their gaseous metabolism tests some further measurements were made on certain organs of nearly one-half of these birds. Very little visible body fat was observed in any bird that survived the 10-day test. Data for "total acetone bodies" in the blood, for liver and muscle glycogen, and for percentage liver fat were obtained at the end of the 10-day fast. Similar additional data were secured by subjecting comparable groups of birds to shorter fasts of only 48 or 120 hours. The methods utilized in this part of the work will be described elsewhere by Riddle and Opdyke in connection with their report upon the action of pituitary hormones in young pigeons.

In an attempt to equalize the nutritive state of the several birds at the beginning of their fast, and to continue our standard method of preparing these birds for heat production measurement 24 hours later, each bird was force-fed enough of a grain mixture to provide its crop with 15 grams of mixed grain and 15 cc. of water. All measurements of the gaseous exchange were made over a period of about 6 hours, at night, in two closed-circuit multiple-chamber apparatus (4), under all conditions earlier recommended by Benedict and Riddle (5) for obtaining the basal heat production of pigeons. These conditions included keeping the birds for 24 hours before measurement in a large glass cage maintained at the same temperature as that at which the birds were to be measured—in this case, at 25° or 30°C. These temperatures were used because it had been learned that, for young pigeons of this breed, the zone of thermal neutrality apparently extends from 25°–30°C.

Only about one-half of our gaseous metabolism measurements included measurement of the carbon dioxide, and this fact influenced our choice of method of calculating heat production in the present series of tests. In each four-chamber apparatus used by us only the two external chambers (extreme right and left) are equipped with the double sets of absorbers required for measurement of CO_2. Thus, though our two similar instruments permitted the simultaneous and independent measurement of the O_2 consumption of 8 birds, we obtained CO_2 values from only 4 of these 8. In all ordinary practice, with birds fasted 24 hours and giving quotients close to 0.72, the (non-protein) quotients obtained from one-half the tests may be safely utilized to determine the heat equivalent of the oxygen used in all of the tests. Under prolonged fasting, however, it was found that the quotients ranged from 0.63 to 0.79, and in this case it is evidently unsafe to assume that any two or four unmeasured quotients are equivalent to two or four that were simultaneously measured. Moreover, the caloric value of oxygen for "protein" quotients (those below 0.70) is not well defined when the nitrogen is burned to uric acid instead of to urea. It is desirable to present these results, like all of our similar studies on pigeons, in terms of heat production rather than as rate of oxygen consumption. For the reasons just noted, however, the oxygen actually used has been assumed throughout to have had the same caloric value (4.686 per liter) as it has in the combustion of fat (R.Q. of 0.70).

It seemed advisable to measure the heat production at both early and late stages of the fast. However, only those hypophysectomized pigeons which had been operated some days before beginning the fast could be tested at the end

of 24 hours of fasting; otherwise recent narcosis, fresh wounds or shock of the operation might affect the measurement.

EXPERIMENTAL RESULTS. *Respiratory quotients.* Examination of the respiratory quotients obtained at the two temperatures (25° and 30°C.) showed that these did not differ significantly. The two groups of quotients are therefore considered together, and their distribution is shown in figure 1. This graph makes it evident that quotients significantly lower than 0.70 were obtained from the second to the tenth days of the fast in both normal and hypophysectomized birds. At all stages of fasting in both types of birds still other high quotients indicate that something besides the burning of fat is involved. Nevertheless, a large proportion of the quotients range from 0.69–0.71 in all brackets, and perhaps it is permissible to consider all or most of these as "fat" quotients (0.70)

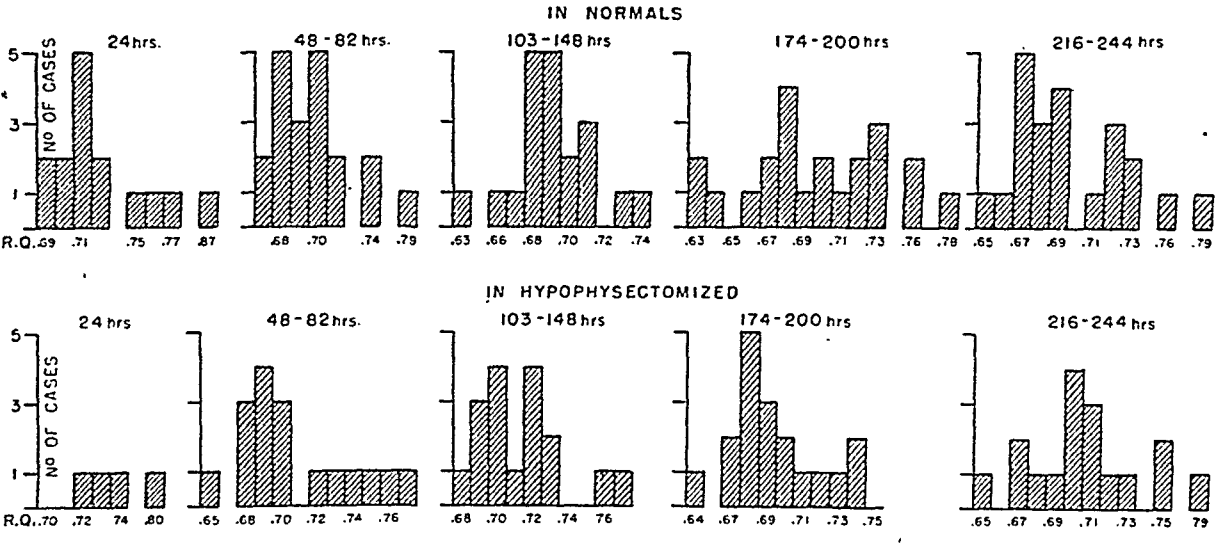

Fig. 1. The value and distribution of respiratory quotients obtained from pigeons fasted during 1 to 10 days.

in which the caloric value of oxygen is 4.686. Again, the data of the graph suggest that the uniform use of a caloric value of 4.686 calories for the oxygen actually consumed, irrespective of the observed quotients, does not introduce any error which would affect unequally the values obtained for the normal and the operated birds.

Some information concerning the course of the respiratory quotients in individual birds should be recorded. In a few birds three quotients were obtained, but for most birds this number was only two. The normal bird which gave the maximum quotient (0.87) at 24 hours, also gave a quotient of 0.73 at 196 hours of fasting. Another normal bird gave quotients of 0.70 at 148 hours, 0.67 and 0.67 at 196 and 244 hours. Still another gave 0.74 at 76 and 0.78 at 196 hours. Finally, a normal bird which gave a quotient of 0.72 at 24 hours, gave a value of 0.63 at 124 hours and 0.69 at 244 hours. Hypophysectomized birds behaved similarly. An operated bird that gave a quotient of 0.72 at 148 hours gave another of 0.73 at 244 hours. Another that gave a value of 0.68 at 74 hours gave

0.64 at 194 hours. Again, a value of 0.65 at 74 hours was followed by 0.74 at 194 hours. The operated bird which gave the extremely high quotient (0.80) at 24 hours, thereafter gave 0.68 at 76 hours, 0.70 at 148 hours, and 0.72 at 196 hours. These data provide some evidence that certain birds in both normal and operated groups were slow in reaching a "fat" quotient, and that perhaps some of them never attained a quotient lower than 0.70 during their 10-day fast. The known loss of body weight by these birds nevertheless would seem to make it certain that all of them metabolized considerable quantities of their own tissue protein.

Stores of carbohydrate and fat. The data of table 1 provide some information concerning changes in the carbohydrate and fat stores in the two types of pigeons during their 10-day fast. These data also indicate that one source of respiratory quotients lower than 0.70 is to be found in the acetonemia which developed in these animals. At the end of a 24-hour fast the hypophysectomized pigeon has a much larger store of liver glycogen than the normal pigeon; this difference completely disappears, however, during the second day of the fast. Though the percentage of liver fat was increased in the fasted birds the "total" liver fat was probably not significantly changed, since livers of birds fasted for 10 days decreased in size (to about 7.0 grams) by 35 to 45 per cent. Not tabulated, but notable, are results of tests on blood sugar during 10-day fasts. In 5 normal birds the average total reducing substances increased from 190 mgm. (second day) to 210 mgm. on the tenth day; in 7 operated birds, injected daily with 10 mgm. of alkaline extract of beef muscle, reducing substances were lowest at 4 days (178 mgm.) but the same (189 mgm.) at 9 days as at 2 days after operation and beginning of fasting.

Heat production. The heat production (calculated from oxygen consumption) and its change during prolonged fasting are indicated in table 2. From the end of the first to the tenth day of the fast the average decrease in rate of heat production in normal pigeons was 28.3 per cent for 4 groups (studied at 30°) and 28.9 per cent for 3 other groups (studied at 25°C.). In the 7 groups of normal birds this decrease varied only from 25.4 to 30.7 per cent; the over-all average of 28.5 per cent may be regarded therefore as an approximately correct measure of the decrease from the basal value caused by nine days of true fasting. The average heat production of the normal birds studied at 25°C. was lower than that of such birds studied at 30°C. by 3.3 per cent at 24 hours and by 3.6 per cent at 244 hours of fasting. The constancy of this difference provides evidence that prolonged fasting did not lead to a shift in the zone of thermal neutrality.

When effects of the environmental temperature are considered it is found that slightly lower rates of heat production, in both normal and hypophysectomized birds, were obtained at 25° than at 30°C. The average heat production of operated birds at 244 hours was higher than that of normal birds by 9.9 per cent when measured at 30°C. and by 6.2 per cent when measured at 25°C. This essentially equivalent result at the two temperatures, together with similar observations on normal birds noted above, provides some evidence that neither

prolonged fasting nor hypophysectomy produces a shift in the zone of thermal neutrality in pigeons.

The heat production is expressed in the table in terms of weight. Certain data concerning actual body weights, and changes in body weight during the fast, are recorded here. Since body weights obtained at the end of a 24-hour fast are more accurate, and since this also was the time of the first measurement of heat production, it may be noted that the 27 normal birds tested at 30°C. then had an average body weight of 451.5 grams; 9 days later their average weight was 358.5 grams. The body weight of the operated birds studied at 30°C. was too variable to express as an average (or to be really informative) at either the beginning or the end of their 24-hour fast, because some of them were operated

TABLE 1

Data for acetonemia, glycogen and liver fat in long-term fasts of young Carneau pigeons (data of Riddle and Opdyke)

	NUMBER OF HOURS FASTED	NUMBER OF BIRDS	BLOOD "ACETONE"	GLYCOGEN		LIVER FAT
				Liver	Muscle	
			per cent	*per cent*	*per cent*	*per cent*
Normal	24	23	1.7	0.55	1.30*	4.32
	48	5	5.2	0.34		5.30
	120	5	15.5			
	240	10		0.36	1.08	5.40
	254	8	13.9	0.27†	0.85	7.85
Hypophysectomized	24	11	4.3‡	2.62	1.00*	3.41
	48	5		0.36		4.90
	216	7		0.08	0.83	
	254	8	12.6	0.16§	0.94*	6.82

* Only 5 birds measured.
† Value obtained from 12 birds.
‡ Only 4 birds measured.
§ Value obtained from 16 birds.

on that or the following day while others had already lost much weight as a result of pituitary removal 2 and 10 days previously. At the end of their fast, however, the average weight of the 23 operated birds was 359 grams. The 12 normal pigeons which were measured to the tenth day at 25°C. had an average body weight of 439 grams at the end of the first day and a terminal weight of 345 grams. The 12 operated birds likewise measured at 25°C. to the tenth day then had an average body weight of 361 grams.

During the last nine days of their fast 39 normal birds showed an average loss of 20.3 per cent of their body weight (as measured at the end of a 24-hr. fast), and in 10 days they lost 27 per cent of their pre-fasting weight. Benedict's (6) fasting man (subject L.) lost 19 per cent in 31 days. From the end of the third day (weight 425 grams) to the end of the tenth day (weight 356 grams) 35

TABLE 2

Effect of fasting and of fasting combined with hypophysectomy on the respiratory metabolism (Cal./kilo/hour) of young Carneau pigeons

NORMAL OR HYPOPHYSECTOMIZED PIGEONS	NUMBER	CALORIES AT VARIOUS HOURS OF FASTING				
		24	48–82	103–148	174–200	216–244
At 30°C.						
Normal	8	4.21*	3.65	3.38	3.13	3.04
Hypophysectomized†	8		3.42	3.24	3.30	3.29
Normal	4	4.28	3.60	3.15	3.25	3.04
Hypophysectomized	1	2.93‡	2.91	3.29	3.24	3.24
Normal	3	4.47*	4.58		3.18	3.13
Hypophysectomized§	3		3.69		2.84	2.74
Normal	8	4.61	3.78	3.60	3.45	3.37
Hypophysectomized†	7		3.75	3.54	3.69	3.72
Normal	4	4.24		3.44	3.10	2.94
Hypophysectomized¶	4			3.71	3.77	3.86
Avg. Normals	27	4.37*	3.81	3.43	3.28	3.13
Avg. Hypct.	23		3.55	3.44	3.44	3.44
At 25°C.						
Normal	4	4.23	3.92	3.28	3.08	3.16
Hypophysectomized	2	3.28**	3.29	3.30	2.95	
Normal	4	4.15*	3.40	3.39	3.50	
Hypophysectomized	2		3.59	3.25	3.65	3.23
Hypophysectomized	2	3.53††			3.45	3.15
Normal	8	4.26*	3.61	3.25	3.09	2.95
Hypophysectomized†	8		3.58	3.45	3.37	3.22
Avg. Normals	16	4.23*	3.63	3.29	3.19	3.02
Avg. Hypct.	14		3.53	3.39	3.35	3.21

* Value is not for the birds which were fasted for 10 days, but the average for all birds (20–94 birds per group) of this type measured at the same season and temperature.

† Fasting started 1 day before operation.

‡ Fasting started 9 days after operation.

§ Fasting started on day of operation.

¶ Fasting started 2 days before operation. Before operation the 24-hour fasting metabolism was 4.08 cals./kilo/hour.

** Fasting started 8 days after operation.

†† At 33 hours after hypophysectomy and beginning of fast.

normal birds lost 16.2 per cent of body weight; within the same interval the average body weight of 30 operated birds, all of which had been operated at least

one day earlier, lost 14.8 per cent of their body weight (from 420 grams to 356 grams).

DISCUSSION. Benedict and Lee (7) measured the nitrogen excretion of three adult geese (4 kgm. body weight) during a long-term fast and made a monographic study of heat production in geese. At 20 to 22 days of fasting protein supplied only 4 to 7 per cent of the total calories produced. Fisher (8) found that 42 to 50 per cent of the nitrogen ingested by homer pigeons is excreted as uric acid. Henry, Magee and Reid (9) fasted fowls for periods up to 7 days and observed increases in the blood sugar at 72 and 96 hours and questionably higher percentages of liver glycogen at these same periods; blood uric acid was fairly constant up to 96 hours and thereafter rose steadily to the end of the fast. Ketone bodies were never found in the urine of these fowls. The respiratory quotient fell to 0.695 at 22 hours and to 0.665 at 35 hours, but was 0.69 at 45 and 49 hours; for the remainder of the fast it was never higher than 0.68 except at 157 hours when it was 0.69. These authors also demonstrated that when their fasting hens metabolized extraneous protein (egg-white, casein, fish meal) quotients greater than 0.697 were never obtained. They further supplied a useful calculation showing that when alanine is burned to urea it gives a quotient of 0.833, but when burned to uric acid a quotient of 0.667. This study was not concerned with the effect of fasting on changes in heat production.

Barott and others (10) made accurate measurements of heat production and gaseous exchange in fasting young fowl, but their studies extended only to the end of the third day. The respiratory quotients (basal) obtained during the second half of this period averaged 0.719 ± 0.004. These authors maintain that the thermal quotients simultaneously obtained by them indicate that, even at this very early stage of fasting in fowl, foodstuffs were being burned at a ratio of 5 per cent carbohydrate, 10 per cent fat and 85 per cent protein. This, however, is not in good accord with other data (9, 11) on the course of nitrogen excretion in fasting fowl. Barott estimated the respiratory quotient of protein in birds at 0.705, and the thermal quotient of oxygen at 3.018 (3.279 for fat), but in view of various uncertainties these values have not been utilized in the present study.

From their study on fasting geese Benedict and Lee (7) concluded that fasting quotients deviating significantly from 0.70 would not be found in careful measurements on geese; and in the many experiments in which they measured only the carbon dioxide, the heat production was calculated on the two assumptions that the respiratory quotients were 0.70 and that they represent a combustion of fat. In the present study the heat production values have been calculated on this same assumption, but in the case of the pigeon we would clearly state our opinion that—excepting values obtained at 24 hours—this procedure is not justified by fact, but only by necessity. For later stages of fasting we do not know the extent to which protein was burned, nor its oxygen equivalent when it was burned. Though the degree of ketonemia observed in these tests on pigeons would tend slightly to reduce the respiratory quotients below 0.70, wholly similar low quotients were observed in fowls (9) in which ketonuria was not present.

The very low quotients obtained from fowl embryos on and near the ninth day of incubation, and its probable explanation in terms of protein combustion, deserve mention. Barott (12) got only quotients of 0.59 or 0.60 from 9-day embryos, and after the eleventh day his values ranged from 0.64 to 0.69. Though Barott suggested that these low values can be explained by assuming a transformation of fat or protein to sugar, it seems more probable that protein combustion was at least partly involved. Needham (13) had earlier found that "the intensity of production of urea, and of uric acid, and the intensity of combustion of protein have all been shown to be greatest from the seventh to the eleventh day of (chick) development." We believe that the 52 respiratory quotients ranging from 0.63–0.68 obtained in the present series of fasting pigeons, together with the important results from adult fowls by Henry, Magee and Reid (9) and from fowl embryos by Needham and by Barott, indicate that birds tend to produce quotients of this order during the combustion of their own or other protein.

During the last half of their fast it was observed that the hypophysectomized birds consumed more oxygen and lost slightly less of their body weight than did the normal birds. Some pertinent observations suggest that this result can be attributed neither to edema nor to increased body temperature in the operated birds. A more probable explanation is that, through secondary atrophy of the thyroid gland, hypophysectomy decreases the endogenous protein catabolism of the operated animals (15); this would involve a more complete utilization of the fat reserves of the operated birds and would enable them to produce more heat from a smaller amount of their own tissue.

The gradual decline of heat production observed in normal pigeons in these tests is in no way unusual, but it is surprising to find that hypophysectomy minimizes the ability of fasting to reduce the rate of oxygen consumption. Hypophysectomy alone, like fasting alone, is generally regarded as a powerful depressant of metabolic rate. At 10 to 20 days after pituitary removal (during which the voluntary food intake was much reduced) the heat production of adult pigeons was observed (14) to decrease by 33 per cent. In the present tests fasting alone was observed to reduce the post-absorptive heat production of normal young pigeons by 28.5 per cent; and, assuming that prior to their operation the operated birds had the same heat production as the normals, calculations show that fasting combined with hypophysectomy resulted in an average reduction of only 22.3 per cent.

SUMMARY

Repeated measurements of the respiratory exchange were made under basal conditions, at both 25° and 30°C., on 43 normal and 37 hypophysectomized young Carneau pigeons subjected to a 10-day fast. Neither prolonged fasting nor hypophysectomy caused any apparent shift of the zone of thermal neutrality.

Fasting was accompanied by a moderate acetonemia, by a slight reduction of both liver and muscle glycogen below the 24-hour fasting level, by little or no change in the total store of liver fat, and probably by no decrease in total reducing substances in the blood.

Respiratory quotients of 0.63 to 0.68 were frequently obtained from both types of birds that had fasted for 48 or more hours; a few quotients of 0.74 to 0.79 were obtained to the end of the fast. The many low quotients obtained in these tests on pigeons, together with available information from earlier studies on fowls, support the view that birds tend to produce quotients lower than 0.69 from combustion of their own protein.

The caloric value of oxygen utilized in the burning of protein by birds has not been determined with acceptable accuracy. On the assumption that the oxygen used had a value of 4.686 calories per liter, irrespective of the respiratory quotients obtained, the heat production of 39 normal pigeons was reduced from a 24-hour fasting value of 4.33 to 3.10 calories per kilo per hour; this is a reduction of 28.6 per cent of the basal value by nine additional days of fasting. Hypophysectomized fasting pigeons showed a comparable decrease of only 22.3 per cent.

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ACID HUMORAL CONTROL OF HEART BEAT[1]

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Received for publication January 21, 1944

Theoretically the acid-humoral mechanism of stimulation proposed for the central nervous system (Gesell, Brassfield and Hamilton, 1941, 1942; Gesell, Brassfield and Hansen, 1942) should hold for all cholinergic systems wherever they exist. These experiments represent an enquiry into the probability of such extension.

According to the acid-humoral concept, acetylcholine functions as the excitatory intermediary, whereas acid acts as a physiological anti-cholinesterase modifying the rate of destruction of the excitatory agent. As an integral part of this mechanism, intensity and duration of stimulation (i.e., strength and duration of electro-tonic flow, Gesell, 1940) are believed to depend upon two factors: 1, the sum total of acetylcholine released at activated nerve terminals, and 2, the rate of destruction of this acetylcholine. A weak and fleeting current might produce but a single discharge of a nerve cell, or a single twitch of a muscle fiber; a stronger and more prolonged current would produce a multiple response. In fact the prolongation of reflexogenic "after-discharge" (i.e., after-hyperpnea resulting from faradic stimulation of Hering's nerve) observed during artificial hypercapnia, combined with the well-known anti-cholinesterase activity of acids, was the evidence upon which the acid-humoral concept originated (Gesell, Brassfield and Hamilton, 1941, 1942). But inasmuch as the central nervous system was not acknowledged as a cholinergic system (experiments on respiration offered little support) the validity of the acid-humoral concept rested on a most critical issue—the existence of humoral intermediation in nerve centers.

It was reasoned, in enquiring into this question, that if acid metabolites function collectively as an endogenous and physiological anti-cholinesterase, a foreign and exogenous anti-cholinesterase might produce changes in breathing comparable to those produced by hypercapnia. Approximate duplication of hypercapnic hyperpnea and potentiation and prolongation of respiratory reflexes by appropriate administration of eserine and of exogenous acetylcholine (Gesell, Hansen and Worzniak, 1943; Gesell and Hansen, 1943) have supported this hypothesis and thereby unified our findings into a more complete and consistent argument for humoral and acid-humoral intermediation in nervous centers.

For the purpose of enquiring into the general biological significance of the anti-cholinesterase activity of endogenous acids several cholinergic systems are under study (Brassfield and Gesell, 1942). Special advantages offered by the heart as compared with the central nervous system are the simplicity of its structure and function, the detailed knowledge of its innervation, and the un-

[1] Preliminary reports, Federation Proceedings, 1943, 1, 10 and 29 and 58.

conditional inclusion of its parasympathetic innervation among the cholinergic systems.

METHODS. Our experiments are based on those of Loewi (1921) in which the perfusate of a vagally inhibited heart of the frog was found to transfer humoral inhibition upon a second and completely isolated heart by virtue of the acetylcholine liberated during the period of vagal stimulation. With these facts in mind our experiments may be divided into two main groups: 1, the effects of acid upon "exogenous" humoral intermediation (i.e., of acetylcholine applied to the heart from without), and 2, the effects of acid upon "endogenous" humoral intermediation (i.e., of acetylcholine physiologically released at the endings of activated nerve fibers). In the first group of experiments the heart (in this case of the turtle) is excised and arranged in a specially constructed glass vessel, open above for recording ventricular contractions, and fitted below with a three way filling and draining stopcock for changing the surrounding environmental solutions. In the second group of experiments the heart is left in situ and exposed sufficiently by an opening in the plastrum and pericardium to permit registration of auricular and ventricular contraction and flooding the heart with the solutions under study. The turtle's head was crushed, rather than pithed, to avoid excessive hemorrhage and to preserve a normal circulation through the heart. The vagus nerves were exposed, freed, cut, and drawn through a combination moist chamber and electrode holder (T tube construction). This arrangement assured a continued dependable uniformity of excitability of the nerve to faradic stimulation.

RESULTS. 1. *Potentiation of exogenous humoral intermediation by simultaneous acidulation with exogenous acid.* For reasons unknown, individual hearts showed considerable variability of sensitivity to the inhibiting action of acetylcholine. Consequently the concentration of acetylcholine required to produce a perceptible slowing of the heart was determined in advance for each experiment. Three solutions were then prepared: 1, plain Ringer's solution; 2, plain Ringer's solution plus acetylcholine, and 3, plain Ringer's solution plus a similar amount of acetylcholine plus carbon dioxide. The heart was routinely exposed to these solutions in the order 1, 2, 1, 3, 1, 2, 1 (see figs. 1A, 1B and 1C). The draining and flooding of the heart, required to change solutions, produces abrupt irregularities which may be disregarded for the tracings are intended to show changes in frequency only. With this in mind, it is clear that the inhibition of the pace setter, produced by carbonated acetylcholine solution at B is considerably greater than that produced by the noncarbonated acetylcholine solution at A and C. The pH's of the noncarbonated and carbonated solutions as determined by the glass electrodes were 7.2 and 5.7 respectively.

Assuming that carbon dioxide and acetylcholine enter the heart freely and simultaneously by the process of simple diffusion, the acetylcholine which enters the tissues from the carbonated acetylcholine containing solution is presumably destroyed more slowly than under ordinary conditions of acid-base equilibrium. It, therefore, attains higher concentrations and exercises greater inhibitory action.

Potentiation referable to inequality of the acetylcholine content of solutions 1 and 2, due to inequality of the rate of its destruction, is ruled out on three

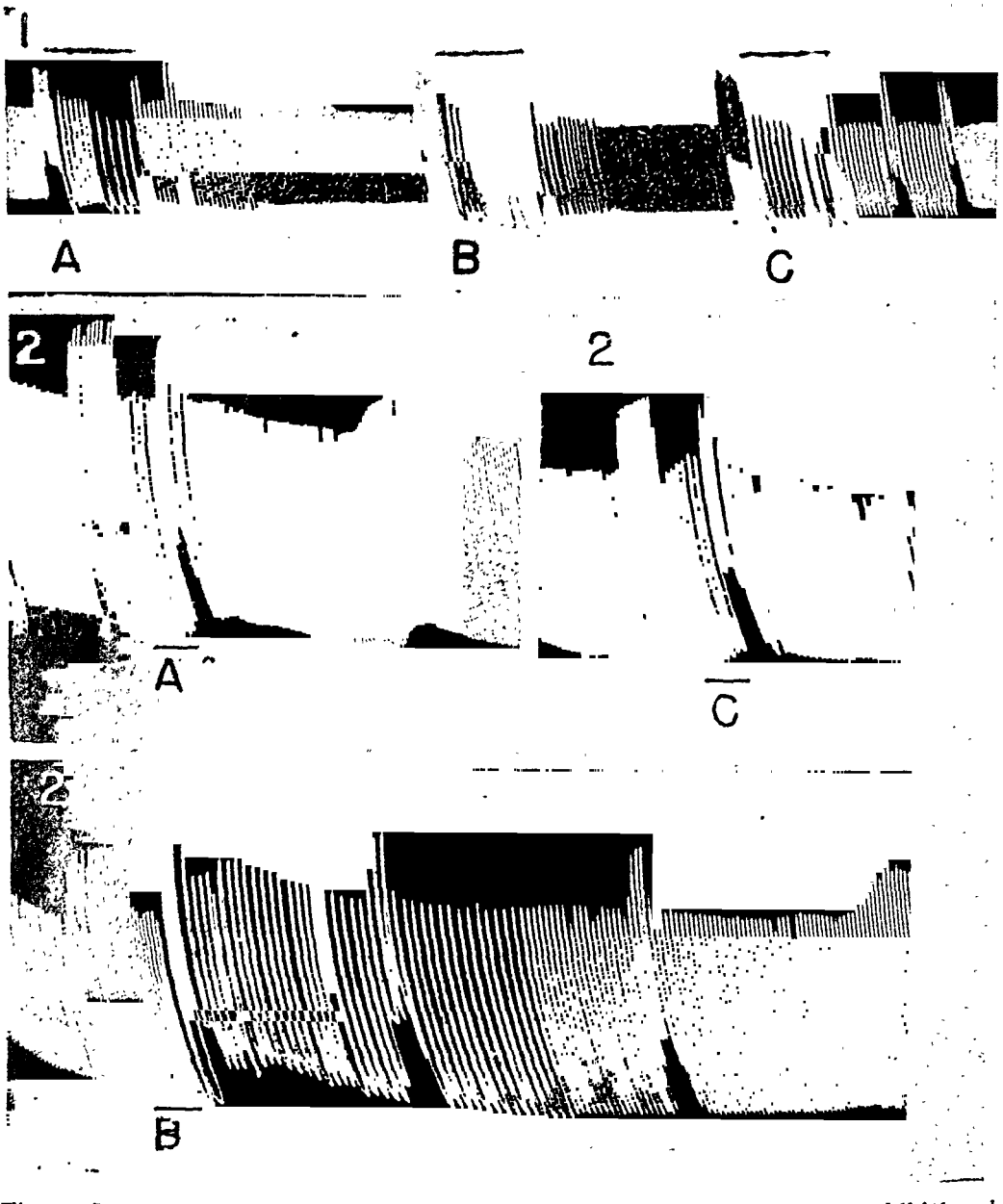

Fig. 1. Potentiation of exogenous humoral intermediation of cardiac inhibition by simultaneous acidulation of the heart with exogenous acid. Ventricular tracing. The heart was covered with acetylcholine containing Ringer's solution at A and C and with acidulated acetylcholine solution at B.

Fig. 2. Potentiation of exogenous humoral intermediation of cardiac inhibition by pre-acidulation of the heart with exogenous acid. Ventricular tracing. The heart was covered with acetylcholine containing Ringer's solution for a period of 20 seconds at A, B and C. Preceding A and C the heart was flooded with plain Ringer's solution. Preceding B it was flooded for two minutes with carbonated Ringer's solution.

points: 1, acetylcholine disappears very slowly in cholinesterase-free solutions at the pH's employed (see Dale and associates); 2, all acetylcholine solutions

were freshly prepared before each test allowing very little time for destruction, and 3, potentiation occurs after pre-acidulation as well as during simultaneous acidulation of the heart (see following section).

In the interest of exactness it is well to appreciate that the term "potentiation" as generally employed in relation to the physiological action of anti-cholinesterases does not conform with the definition "to render more active physiologically" (Webster), for so far as we are aware neither acid nor eserine serve to increase the physiological response to acetylcholine. While it is conceivable that carbon dioxide might exert an effect independent of its anti-cholinesterase action, our figures give no evidence on that point. When acting by itself there is little or no effect on rhythmicity of the heart (compare the beginning of figs. 3A and 3C with 3B). This agrees with Smith (1926) that rhythmicity of the turtle's heart is maintained with remarkable constancy under great changes of carbon dioxide. However when carbon dioxide works jointly with acetylcholine it exercises remarkable effects. It is, therefore, believed that carbon dioxide and acetylcholine enter the heart freely and simultaneously by a process of simple diffusion, and that the acetylcholine which enters the tissues from the carbonated acetylcholine containing solution is destroyed more slowly than under ordinary conditions of acid-base equilibrium. It consequently attains higher concentrations and produces greater inhibitory action.

2. *Potentiation of exogenous humoral intermediation by pre-acidulation with exogenous acid.* These pre-acidulation experiments have a special significance of contrasting the rôle of tissue pH with that of the acetylcholine carrying solution in which the heart is placed. That point is illustrated in figures 2A, 2B and 2C. First in order of procedure (see fig. 2A), the normal beat is established by flooding the heart with plain Ringer's solution. The heart is then drained and covered with acetylcholine containing Ringer's solution for a period of 20 seconds as indicated by the horizontal bar to record the degree of inhibition occurring under so-called normal acid-base conditions. Next the heart is reflooded with plain Ringer's solution which reveals the rate of recovery and the after-inhibition which may be analogous to the "after-discharge" seen in the central nervous system after reflex stimulation. Second in order of procedure (see fig. 2B) the heart is exposed for several minutes to a carbonated Ringer's solution, free of acetylcholine, for the purpose of pre-acidulating the thin-walled structures of the basal part of the heart where the parasympathetics terminate. The pre-acidulated heart is then re-exposed to the acetylcholine-containing and carbon dioxide-free solution precisely as in figure 2A. It is then reflooded with plain Ringer's solution and allowed to recover. In figure 2C all procedures of figure 2A are duplicated for the final control. The greater degree of cardiac inhibition as a result of the exposure to acetylcholine following pre-acidulation of the heart and the greater extension of this inhibition into the period of recovery manifests the potentiation of exogenous humoral intermediation by pre-acidulation.

Comparable potentiation by simultaneous acidulation (fig. 1) and by pre-acidulation (fig. 2) indicates that tissue acidity is the essential factor involved in potentiation and that the order of entrance of acid and acetylcholine into the

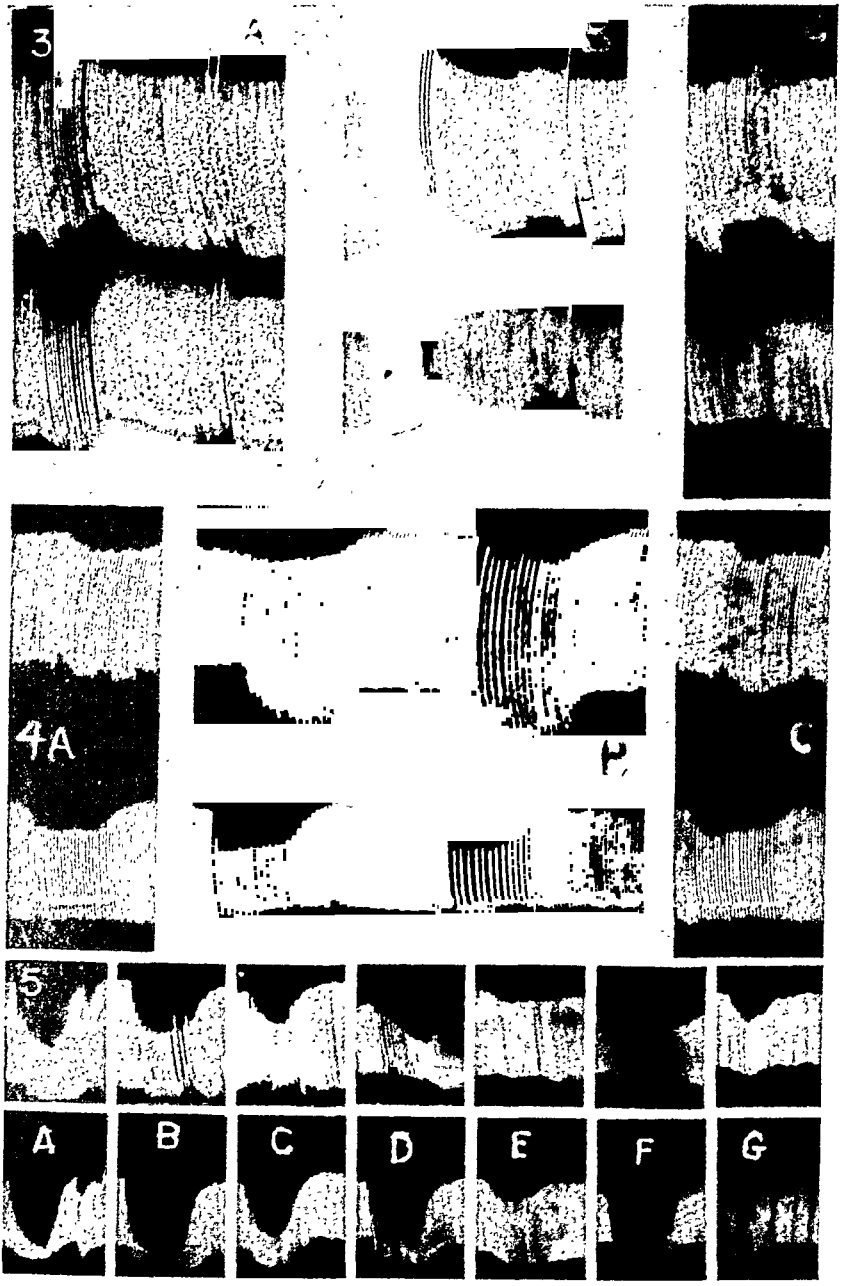

Fig. 3. Potentiation of vagal inhibition of the heart (i.e., endogenous humoral intermediation) by exogenous acid. Ventricular tracing above and left auricular tracing below. The heart was covered with plain Ringer's solution for several minutes preceding stimulation in figures 3A and 3C. Preceding stimulation in figure 3B it was covered with carbonated Ringer's solution.

Fig. 4. Potentiation of vagal inhibition of the heart (i.e., endogenous humoral intermediation) by endogenous acid. Ventricular and left auricular tracings. Before vagal stimulation at A and C the heart was flooded with plain Ringer's solution for a period of several minutes. Before D the heart was flooded with a sodium cyanide containing Ringer's solution for a similar period.

Fig. 5. Additive potentiation of vagal inhibition of the heart by the combined action of exogenous and endogenous acids. Left auricular tracing above, right auricular tracing below. The right vagus nerve was stimulated. In the control observations A, C, E and G the heart was covered with plain Ringer's solution. B and D show the individual potentiating effects of carbon dioxide and sodium cyanide respectively. C shows the combined potentiating effects of these acids.

tissues is of minor, if any, importance. The prolonged "after-inhibition" or after-discharge during the recovery shown in figure 2B agrees with a slowly progressing de-acidulation and a slowly diminishing anti-cholinesterase activity of tissues.

3. *Potentiation of "endogenous humoral intermediation" by exogenous acid.* To investigate this possibility the vagus nerve (either left or right) was stimulated at regular intervals with a faradic current just strong enough to produce a minor retardation of cardiac rhythm and a slight reduction of amplitude of auricular contraction. The heart in turn was covered in alternate order with noncarbonated and carbonated Ringer's solution before each stimulation. The right vagus nerve was stimulated in the experiments illustrated in figure 3 for periods of 20 seconds' duration and contractions of the ventricle and left auricle recorded. The heart was covered with plain Ringer's solution in figures 3A and 3C and with a Ringer's solution saturated with a 20 per cent carbon dioxide mixture in figure 3B. The striking augmentation of the chrono- and inotropic effects of vagal stimulation by carbon dioxide indicate that endogenous humoral intermediation can be potentiated by exogenous acid in a way similar to the potentiation of exogenous humoral intermediation. As far as we can see these results are analogous to the potentiation of reflexogenic hyperpnea in the respiratory centers produced by hypercapnia (Gesell, Brassfield and Hansen, 1942).

4. *Potentiation of endogenous humoral intermediation by endogenous acid.* The similarity of exogenous and endogenous humoral intermediation of cardiac inhibition combined with the potentiation of each by exogenous acid bespeak a freedom of movement of humor and acid to their site of action in the tissues of the heart, and suggest the probability that potentiation of humoral intermediation may also occur under physiological conditions entailing an increased production of endogenous acid. This hypothesis was tested with the aid of sodium cyanide, a substance known to increase the lactic acid content of living tissues. The vagus nerve was stimulated at regular intervals as described in the preceding section and the heart alternately covered with plain Ringer's solution and Ringer's solution modified by the addition of sodium cyanide (see figs. 4A, 4B and 4C). Figures 4A and 4C, where the heart was flooded with plain Ringer's solution, serve as the controls to the two observations in figure 4B where the heart was subjected to the action of sodium cyanide (0.0004 M). As in figures 3A, 3B and 3C the right vagus nerve was stimulated (20 sec.) and the contractions of the ventricle and left auricle were recorded. Greater diminution of frequency and strength of auricular contraction and prolongation of after-discharge in figure 4B indicates potentiation of humoral intermediation by endogenously formed acid.

5. *Additive potentiation of endogenous humoral intermediation by exogenous and endogenous acid.* In attributing the potentiating effects of exogenous and endogenous acids to a modification of tissue pH it is implied that their effects are additive. This implication seemed worthy of study. The method used is essentially that described for figures 3 and 4. Results of one set of observations in which the right vagus nerve is stimulated and the left and right auricular con-

tractions recorded are shown in figures 5A to 5G. Figures 5A, 5C, 5E and 5G are the controls showing the effects of uniform vagal stimulation while the heart is covered with plain Ringer's solution. Figures 5B, 5D and 5F show the intervening effects of similar stimulation while the heart is covered, first with carbonated Ringer's solution equilibrated with a 20 per cent carbon dioxide gaseous mixture, second with Ringer's solution to which is added cyanide, and third with Ringer's solution to which both carbon dioxide and cyanide are added. Either carbon dioxide or cyanide alone have but a small potentiating effect upon vagal inhibition of frequency and strength of beat; when combined the effect is decidedly greater. The inference is that the individual pH effects of the exogenous and endogenous acids are additive. The possibility that cyanide may contribute additional anti-cholinesterase activity by direct poisoning of the cholinesterase is not excluded.[2]

Discussion. There are several reasons for believing that potentiation of humoral intermediation by acid may possess a broad biological significance. Most important is the occurrence of potentiation in all supposedly cholinergic systems so far tested with carbon dioxide—the central nervous system, the heart, respiratory muscle, the intestinal loop and the submaxillary gland. The demonstration of potentiation of cardiac inhibition by lactic acid as well as by carbon dioxide point to pH as the instrument involved. By virtue of this common integrant, the miscellaneous acid products of tissue metabolism would be empowered to combine their effects into a fluctuating anti-cholinesterase activity and conceivably play an integrative rôle of no mean importance. Those acids which are produced in great and in fluctuating quantity, notably carbonic and lactic acids, would be expected to play a major part in the control of cholinesterase activity in the tissues. The freedom of diffusion and of gross transport of these acids from one neighboring locus to another, or from one distant tissue to another would contrive to make them function locally and generally. The increase in acidity resulting from an excessive formation of carbon dioxide in hyperactive tissue, or from an excessive formation of lactic acid in augmented anaerobic metabolism could therefore provide a co-ordinated and automatic adjustment to changing respiratory or energy requirements. Direct experiments by Winder (1942) have shown that the local stimulating effects of carbon dioxide excess and of oxygen lack in the carotid body are additive. Since v. Euler, Liljestrand and Zotterman (1941) "look upon the sinus region as a sort of nervous center with peripheral localization of the same general type as in the olfactory or optical peripheral organ" it was proposed (Gesell and Hansen, 1943) that the additive effects noted by Winder are an expression of their combined anti-cholinesterase activity, i.e., pH. In the present paper we add a second example of such additive effects of carbonic and lactic acids in the potentiation of the response of the heart to vagal stimulation.

Scattered and miscellaneous observations in the literature would seem to lend support to our views. Direct agreement with our experimental results is found in the experiments

[2] Suggested by Doctor Cantoni.

of Andrus (1924) on the effects of certain changes in the perfusate upon the isolated auricle of the rabbit, viz. "The inhibitory effects of certain vagomimetic substances—acetylcholine and choline—upon the auricle is more pronounced at PH 7 than at PH 8. Stimulation of the vagus of the tortoise is likewise more effective in stopping the heart at PH 7 than at PH 7.8." Segers (1939) found that sodium bicarbonate diminished the inhibitory effects of vagal stimulation in the frog.

The relation of intensity of vagal inhibition of the heart of the turtle to the amount of acetylcholine liberated in the heart is established by Brown and Eccles (1934A). They find the curve of inhibition of the "left vagus to be indistinguishable, except for intensity, from that of the right vagus which is stronger" and show that eserine increases the intensity and prolongs the inhibition of a single volley. Hence it is concluded (agreeing with the effects of acid in our experiments) that the decline of the inhibitory curve is due to the progressive destruction of the liberated acetylcholine by cholinesterase. Additive effects of single volleys to the left and right vagus are demonstrated by the agreement of the theoretically combined inhibitory curve with that of actual combination of left and right vagal stimulation. To quote (Brown and Eccles, 1934B) "Since as was shown above, the right and left vagus nerves are distributed independently to the pace maker, a volley down each would produce a concentration of acetylcholine substance equal to the sum of the concentration produced by either volley alone." These additive effects of individual "liberations" of acetylcholine may be regarded as analogous to those of individual "preservations" of acetylcholine demonstrated for the combined anti-cholinesterase activity of carbonic and lactic acid. Greater potentiation of vagal inhibition by the combined effects of eserine and carbon dioxide, as compared with that of either alone, illustrate the additive effects of preservation of acetylcholine by two widely differing anti-cholinesterases (Gesell, Brassfield and Hansen, 1942).

According to Brown and Eccles (1934B) the latent period of inhibition of the heart by a single volley is 100σ. This leads them to believe that most of the latent period is consumed in diffusion of acetylcholine from the site of liberation to the site of action, a conclusion of interest to the freedom of movement of acid metabolites and of neurohumors expressed in our paper.

Feldberg and Gaddum (1934) express the opinion in their study on the chemical transmitter at synapses in a sympathetic ganglion that it is "even probable that the preganglionic vagus fibers are also cholinergic and since their ganglionic synapses lie in the heart tissue their mechanism may also make some contribution to the Vagusstoff." Consequently both pre- and post-ganglionic terminals must be considered in acid-humoral control of heart beat. The findings of Bronk and Larrabee (1937) on the effects of activity and altered circulation on ganglionic transmission are interesting in that connection for they may be ascribable to the greater accumulation of endogenous acids which must occur in the non-perfused ganglion. "In a circulated ganglion submaximal preganglionic stimulation at a frequency of 5 to 10 a second produces a discharge of postganglionic volleys with a corresponding frequency and of a constant size. Ten to fifteen minutes after the circulation has been stopped similar stimulation produces a series of postganglionic responses which progressively increase in size until they become 4 or 5 times as large as the initial volley of the series. Furthermore, if the stimulation be continued for some seconds and then stopped, a preganglionic volley 30 seconds later will still evoke a postganglionic volley larger than the initial test response. Here is evidence for long-lasting facilitation in non-circulated ganglia."

Cannon and Haimovici (1939) state that "The simplest way to excite spinal nerve cells is by partial asphyxia." This was done by stopping artificial respiration rather than circulation. Bauer (1938) in his experiments on the effect of asphyxia upon the heart rate of rabbits at different ages states that "Stimulation experiments show that a stimulus which is above threshold for an 11-day rabbit fails to slow the heart at earlier ages under ordinary conditions but will do so if the animal is asphyxiated." Porter, Blair and Bohmfalk (1938) studying facilitation of reflex action in the spinal cat find with a method capable of detecting

the entrance of individual motor units—1. Slight asphyxia, caused by the intravenous injection of sodium cyanide, increases the number of motor units responding to the same sensory stimulus as before. 2. The increased response is not a direct stimulation of motor neurons because no contraction occurs if sensory stimulation is discontinued during the period of increased contraction. 3. Asphyxia produced by increased intraspinal pressure acts similarly. 4. Neither injection of cyanide nor increased intraspinal pressure causes new motor units to enter into the contraction when the tenuissimus is used as a nerve muscle preparation.

Many other observations might be cited suggesting that acid control of humoral intermediation may play a broad biological rôle.

SUMMARY

The phenomenon of "potentiation" of humoral intermediation by acid was studied on the heart of the turtle.

It was found that carbon dioxide added to an acetylcholine containing Ringer's solution augmented the inhibitory effects of this solution. This may be regarded as an example of potentiation of exogenous humoral intermediation by an exogenous acid.

Pre-acidulation of the heart by immersion in carbonated Ringer's solution was found to produce similar potentiation which indicates the significance of tissue acidity.

Exposure of the intact heart to carbonated Ringer's solution increased the inhibition produced by faradic stimulation of the vagus. This finding is regarded as an example of potentiation of endogenous humoral intermediation by exogenous acid.

Exposure of the heart to small concentrations of cyanide in Ringer's solution also increased the effects of vagal stimulation. It is suggested that this result illustrates potentiation of endogenous humoral intermediation by an endogenous acid (i.e., lactic acid).

Simultaneous exposure of the heart to carbon dioxide and cyanide produced a much greater potentiation than exposure to either substance alone. This is thought to indicate an additive effect of two endogenous acids upon tissue pH and anti-cholinesterase activity.

Reasons are briefly stated in support of the hypothesis that potentiation of humoral intermediation by acid may provide an automatic chemical adjustment of general biological interest.

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THE RELATIONSHIP OF COPPER TO HEMATOPOIESIS IN EXPERIMENTAL HEMORRHAGIC ANEMIA[1]

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Received for publication January 21, 1944

Recent reviews on the necessity of dietary copper in animal metabolism (1, 2, 3) have summarized the details of earlier experiments. The general conclusions that copper is necessary as a supplement to iron for hematopoiesis; that of a large group of elements, copper is unique in this respect; and that copper is not concerned with the assimilation of iron but with the conversion of iron to hemoglobin; have been extended to a rat, mouse, chicken, rabbit, pig and lamb.

Since there is no reason to expect a difference in the mechanism of hematopoiesis in the dog and other red blooded mammals, it would seem likely that a hemorrhagic anemia in the dog would respond similarily to the administration of copper and iron. However, Whipple and Robscheit-Robbins (4, 5, 6) using dogs with prolonged hemorrhagic anemia on a salmon bread diet, have been able to obtain only irregular responses to the use of dietary copper.

Earlier experiments in this laboratory also indicated irregular and incomplete responses to copper (7, 8). This difficulty was experienced with both hemorrhagic and nutritional anemia in young dogs. While a prompt response to copper feeding was obtained within two weeks, the hemoglobin level of the blood seldom rose above 10 grams per 100 cc. of blood. Although the animals were on a raw milk diet and were young growing dogs there seemed to be no reason to doubt the adequacy of the ration when supplemented with iron, copper and manganese. Since our early work it has become evident that maximum hemoglobin levels are not reached in the dog until maturity. In most cases on an adequate diet the hemoglobin level increases from a level of 7 to 9 grams per cent immediately after weaning to a level of 11 to 13 grams per cent during the following 2 to 3 months with further gradual increases. Additional work has shown that dogs receiving a casein-sucrose diet supplemented only with the crystalline B vitamins show rapid and complete hemoglobin response after severe phlebotomy (9). The rather high level of B vitamins in the ration compared to the milk diets suggested a possible significant difference in the rations. With this information available, new experiments were undertaken.

EXPERIMENTAL. Eight recently weaned puppies were selected for this experiment. Six of these (2–7) were hound littermates, approximately 7 weeks old

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

Supported in part by a grant from the Wisconsin Alumni Research Foundation.

We are indebted to Merck and Company, Rahway, New Jersey for the synthetic vitamins and to Abbott Laboratories, North Chicago, Illinois for haliver oil.

when started on the diets. Dogs 1 and 8, of collie extraction, were 9 weeks of age. They were all injected with distemperoid and treated twice with an anthelmintic (tetrachlorethylene) before being placed on the experiment.

The dogs were kept in wooden metabolism cages upon galvanized wire screening. The raw milk ration, supplied from earthenware crocks, was supplemented with 100 micrograms thiamine and riboflavin, 60 micrograms pyridoxine, 500 micrograms calcium pantothenate, 2 mgm. niacin, 50 mgm. choline and 3 drops of haliver oil per kilo of body weight per day. Three milligrams manganese as $MnSO_4 \cdot H_2O$ was supplied daily, and copper as $CuSO_4 \cdot 5H_2O$ and iron as $FeCl_2 \cdot 4H_2O$ further supplemented the ration as indicated below.

Body weights and blood samples were taken before the morning feeding. Blood samples for analysis were withdrawn from the radial vein. Routine hemoglobin, hematocrit and red cell counts were made as in previous experiments (10). The dogs were bled from the external jugular vein, removing approximately 20 per cent of the estimated blood volume at a single bleeding. The animals were bled over a three day period to reduce the hemoglobin level to approximately 6 grams per cent, normally considered a severely anemic level.

Results. Dog 2, the positive control, receiving mineral supplementation of 3 mgm. copper and 30 mgm. iron, after an initial decline during the first week, showed a gradual increase in hemoglobin, hematocrit and red blood cells. Dogs 1, 4–7, receiving 30 mgm. iron, but no copper showed a similar response in hemoglobin during the same period. The pups were now $3\frac{1}{2}$ to 4 months old. The animals were bled heavily during the ensuing 3 days reducing the hemoglobin level to 5.1–6.4 grams per cent.

The positive control, to which copper was always available, showed an immediate increase in hemoglobin, reaching 11.6 grams per 100 cc. at the end of 19 days. The administration of 3 mgm. copper to dog 7 immediately after hemorrhage brought about a similar increase in hemoglobin. Dog 6 received no copper for 20 days, during which time the hemoglobin level remained below 5.6 grams per cent. However, within one week after the daily administration of 3 mgm. copper the hemoglobin rose to 7.9 grams per cent and continued to increase to 11.8 grams per cent in the following two weeks. Dog 5 was not fed copper until 27 days after hemorrhage. The hemoglobin fluctuated between 6.4 and 7.5 grams per 100 cc. within that 27 day period but was at 6.8 grams per cent at the time of copper feeding. The administration of 3 mgm. copper daily brought about the usual sharp response during the next three weeks. The administration of copper was delayed for 46 and 48 days in the case of dogs 1 and 4. During this period the dogs exhibited a slow increase in the hemoglobin level for the first four weeks, only to drop again by the end of the period. The hemoglobin response to copper feeding in both dogs was very rapid. The complete changes in the hemoglobin levels for the individual dogs are shown in figure 1.

Dog 8, supplied with 3 mgm. copper and no iron, showed a severe nutritional anemia after 15 days on the raw milk diet. The hemoglobin level reached 4.8 grams per cent. At this point 10 mgm. iron was fed daily. The hemoglobin level rose gradually to a level of 13.1 grams per cent at the conclusion of the

experiment. Dog 3, fed adequate copper, received no iron. The hemoglobin level dropped slowly from the original 8.2 grams per cent to 6.0 grams per cent two months after the start of the experiment. Twenty milligrams iron were then fed daily in addition to 3 mgm. copper and the hemoglobin level rose gradually to 12.1 grams per cent at the end of the experiment.

The red cell count at the start of the experiment ranged from 3.68 to 4.92 million per cubic centimeter for the 5 animals without copper. These counts

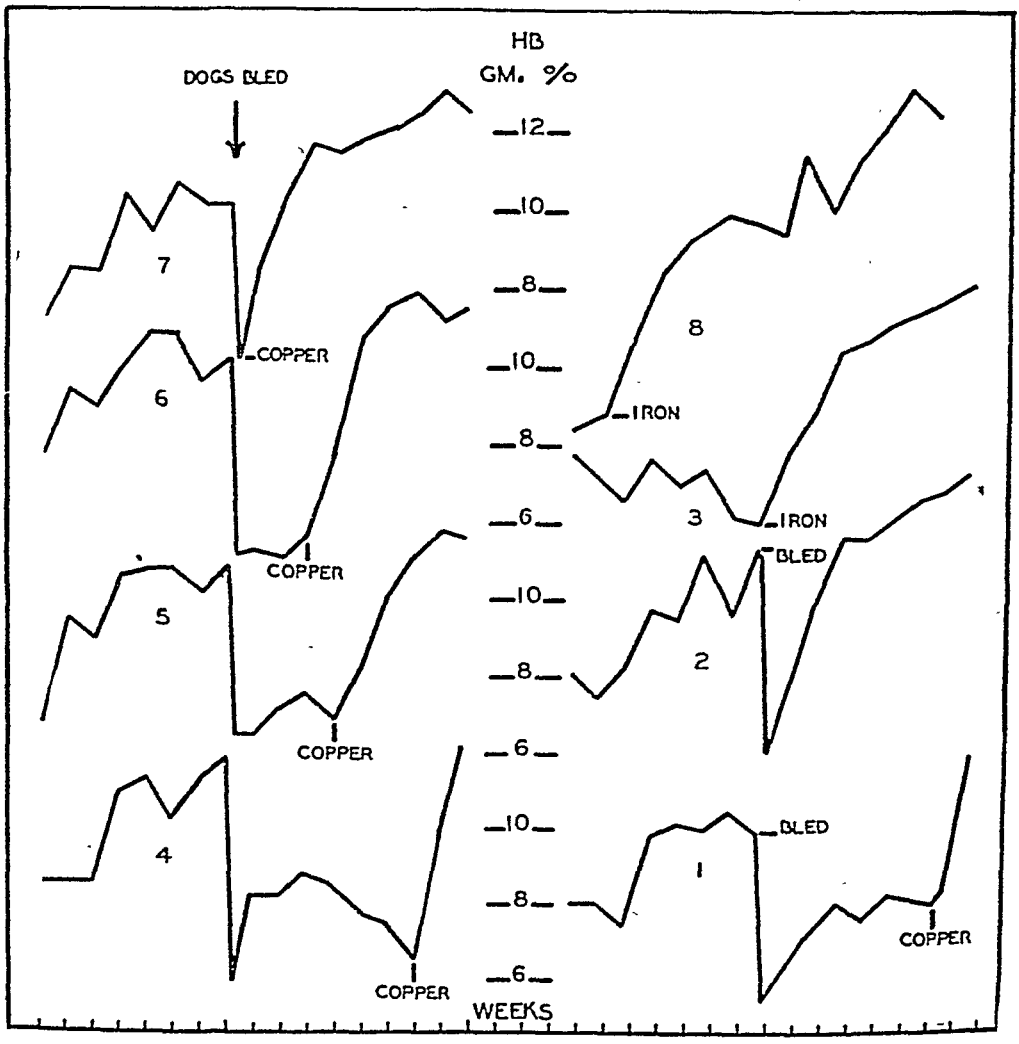

Fig. 1. The complete hemoglobin picture for the individual dogs during the course of the experiment.

had increased to a range of from 4.95 to 6.09 after two months on the experiment and just prior to phlebotomy. The animals were then heavily bled removing 200 to 425 cc. The red cell count dropped sharply and remained at 2.31 to 3.50 million until copper was added. The red cell count in dog 5 remained depressed for one week before exhibiting the sharp response shown by dogs 6 and 7. Dogs 1 and 4 showed a gradual increase in red cells to 3.6 and 4.0 million before copper was fed, and after copper therapy gave a rapid increase in red cell count. Dog 4 exhibited a two day delay in the cellular response. The red blood count of dog 2

rose from an original 4.26 million to 6.23 million just prior to phlebotomy. After bleeding, the count dropped to 3.12 million and remained below 3.0 million for the next five days. Only during the following week was a cellular response obtained that was typical of dogs 6 and 7.

Dog 3 showed no distinguishing characteristics during the iron deficient period. The administration of 20 mgm. iron after the nutritional anemia occurred caused only a gradual increase in cell counts. This was also true of dog 8. The animal had a lower original cell count and responded to the feeding of 10 mgm. iron with a sharp rise in cell count to about five million and remained there thereafter. The graphical representation of all red cell counts is shown in figure 2.

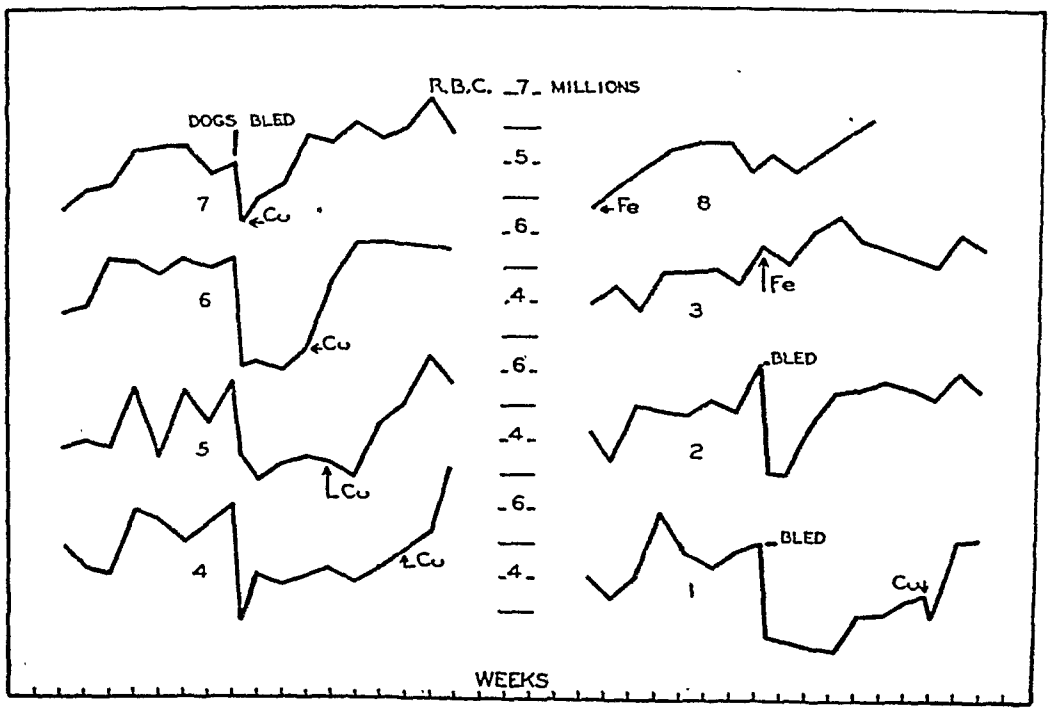

Fig. 2. The complete red blood cell counts for the individual dogs during the course of the experiments.

Copper has apparently some influence on the gross body weight. None of the animals on a copper deficiency showed any deviations in weight increases from the control animal up to the time of phlebotomy. After the animals were bled weight differences did occur. Dog 2, receiving adequate copper, and dog 7, supplied with copper immediately after phlebotomy, gained 4.5 and 5.6 kilo respectively. These values compared with an increase of 0.05 and 1.9 kilo for dogs 1 and 4 for a comparable period. After copper therapy both dogs gained weight rapidly bringing the total weight of the animals approximately equal to that of the positive control. There was little or no increase in weight during the period of one month when dogs 5 and 6 were without dietary copper. The weight responses of these animals to copper resulted in weight increases of 3.5 and 1.5 kilo so that the animals again paralleled the growth of the control dog.

An iron deficiency of two months' duration in dog 3 caused a delay in growth.

In both instances of iron administration to dogs 8 and 3 the weight increases coincided with iron therapy and equalled the growth of the control dog.

In general the appetite remained unaffected in the copper deficient dogs before bleeding. After phlebotomy, however, continued deprivation of copper resulted in a depressed appetite and suppressed growth. There was an immediate recovery of appetite upon the administration of copper to these animals. This poor appetite on the iron deficient ration, and the immediate stimulation after supplementation with iron was also shown by dog 3.

The mean corpuscular volume (calculated) varied widely in all dogs on the copper deficiency. The extreme values ranged from 53 to 73 u^3 with a mean of 63 u^3, which does not differ significantly from the mean values reported in the literature by Leichsenring (11). During the course of phlebotomy the mean corpuscular volume of the control and copper deficient dogs generally increased over the following 2 to 19 days. In two instances (dogs 5, 7) there was a slight decrease over the two day bleeding period which was then followed by a mean volume increase within the next two weeks. The mean cellular volume response to copper therapy was one of general increase, concomitant with the hemoglobin increase. Dog 6, however, showed a delay in this respect; the response did not occur before the second week.

The iron deficiency showed a greater effect on the corpuscular volume. Dog 8 had a cellular volume of only 45–46 u^3 after 15 days on the iron deficient diet. Within two weeks after the administration of 10 mgm. iron the volume increased to 70 u^3. Hereafter, variations resulted in a mean volume of 64.7 u^3 which can be considered normal. Dog 3 exhibited a continual depression of corpuscular volume throughout the iron deficient period, the values decreasing from 62.8 to 36.6 u^3. The administration again brought about normal values.

Changes in the saturation index were insignificant. On the whole the hemoglobin in the cell seems to saturate the cell as long as it exists as a mature cell.

Discussion. Leichsenring (11) has reported a mean value of 17.5 ± 2.96 cc. for the oxygen combining capacity in volume per cent for normal dog blood. Using the commonly accepted value of 1.34 cc. of oxygen per gram of hemoglobin it is possible to convert this value into a hemoglobin value of 13.1 ± 2.21 grams per cent. Values in this laboratory for adult dogs on a complete ration have been found to range from 13.0 to 18.2 grams per cent with a mean of 15.6. These values and the values of Gibson et al., Wintrobe, et al., and others cited by Leichsenring are comparable. There is a greater variation in the hemoglobin level of younger dogs. Values as low as 4.9 grams per cent immediately after weaning and 9.8 grams per cent 4 weeks later, have been observed. It is apparent that the hemoglobin content of the blood rises with the growth of the animal until maturity is reached. This picture differs in no way from that shown in rats (12, 13) and in humans (14). This would indicate that the hemoglobin levels attained by the dogs after the administration of copper are in the normal range.

Robscheit-Robbins and Whipple (15) have stressed the fact that potential hemoglobin production increases with the severity of the bleeding. It is obvious

then that as the normal hemoglobin level of the animal is approached the rate of hematopoiesis should drop off, the stimulus for rapid hematopoiesis being lacking. This is borne out by the fact that the hemoglobin of all the dogs responded rapidly within two weeks after therapy, reaching a hemoglobin level of 11.0–12.0 grams per cent and rose only slowly thereafter to a level of 13.0–15.0 grams per cent.

There are reports in the literature of high blood copper values in chlorosis and pernicious anemia and of a rapid rise of blood copper after both acute and chronic hemorrhagic anemia (7, 16). This increase in the blood is undoubtedly the result of the rapid mobilization of the copper stores from the tissue and presupposes a functional significance in relation to blood formation. On a prolonged diet, the raw cow's milk supplying only 0.10 to 0.20 mgm. copper per liter, there was obviously a gradual depletion of the copper reserves. The original bleeding of these animals then resulted in a mobilization of the available stores and a final depletion of the mobilized copper with the continued bleeding. This technique leaves the dog normal in most respects but has deprived it of copper and hemoglobin.

A comparison of the data of dogs 8 and 3 with the remainder of the dogs shows clearly the complete dependency of the two metals upon each other for adequate blood formation.

SUMMARY

Young growing dogs on a raw milk diet supplemented with crystalline B vitamins and manganese were rendered anemic by bleeding. Blood analysis showed a characteristic picture of secondary anemia.

The anemia showed no response to dietary supplementation of iron in the absence of copper. The daily administration of 3 mgm. copper brought about an immediate and rapid increase in hemoglobin, hematocrit and red cell counts to normal values.

An iron deficiency in the presence of adequate (3 mgm.) copper caused a nutritional anemia and a concomitant decrease in mean corpuscular volume. The administration of 10, 20 or 30 mgm. iron with 3 mgm. copper gave the characterstic remission in the anemic dogs.

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A STUDY OF GELATIN AND SALINE AS PLASMA SUBSTITUTES[1]

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Received for publication January 25, 1944

In previous work (1) it was observed that intravenous infusions of 5 per cent ossein gelatin in 0.9 per cent saline solution, when administered intermittently over a 7 hour period, were effective in preventing the shock which follows release of tight leg tourniquets in anesthetised animals. On the other hand, gelatin similarly administered to dogs subjected to leg muscle trauma, did not result in any greater number of survivals than obtained by employing infusions of an equal quantity of physiological salt solution.

The marked decrease in blood volume following release of leg constrictions is due to leakage of plasma through capillaries injured by prolonged anoxia. However, the deeply anesthetized dog shocked by traumatizing thigh muscles, loses whole blood into the injured area owing to rupture of numerous small vessels. Furthermore, in shock due to this procedure there is present an important nervous factor which contributes to the syndrome.

Since gelatin was effective in preventing one type of shock characterized by plasma loss, it was considered worth while testing it in shock induced by methods other than those previously employed, where plasma rather than whole blood loss into the legs also appears to be the principal initiating factor, and nervous influences are apparently not concerned.

We are indebted to the Upjohn Company of Kalamazoo, Michigan, for supplying us with ample quantities of specially prepared bone collagen gelatin. This gelatin, which has been autoclaved twice in order to increase fluidity, is prepared in physiological saline solution and has an oncotic pressure of 70 mm. Hg after sterilization. It is free from pyrogens, and preserved with phenyl mercuric borate, 1:25,000. We have not detected any ill effects resulting from intravenous use of large quantities of this gelatin, either at the time of injection or later.

Considerable literature has accumulated pertaining to the study of gelatin as a plasma substitute since Hogan (2) reported his results on patients. Gelatin-saline solutions have in recent years been tested in the treatment of various types of experimental shock and have been used on patients with some degree of success. According to most investigators, it is not as effective as plasma but is more efficacious than saline or other crystalloidal solution (3–12).

METHODS. The method adopted for producing shock was that originally de-

[1] We are indebted to the Upjohn Company of Kalamazoo, Michigan for providing funds to defray a part of the expenses of this investigation.

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vised by Duncan and Blalock (13), and consisted of the prolonged application of a leg press, made according to the specifications published by these investigators. Preliminary experiments in which we followed the Duncan-Blalock technique of applying 500 pounds' pressure for 5 hours to one thigh of the animal under deep anesthesia did not prove entirely satisfactory. However, 750 pounds' pressure applied for 7 hours as high on one thigh of the anesthetized dog as possible gave consistent results.

The animals were healthy, mongrel dogs, averaging 10.6 kgm. in weight. They were kept in the laboratory for at least one week before using, for it has been our experience that allowing sufficient time for the dogs to become well adapted to laboratory conditions before administering trauma of any kind, is important in shock studies where survival is used as a test of the efficacy of any therapeutic measure.

Before applying the press, the dogs were given 30 mgm. of morphine subcutaneously, followed within 30 minutes by an intravenous injection of 20 mgm. of pentobarbital sodium per kilogram of body weight. Deep anesthesia was maintained throughout all experiments and at no time was the anesthesia allowed to become light. The hair was removed from the right limb by electric clippers and the entire leg thoroughly cleansed with green soap and bathed with 80 per cent alcohol. The press was then adjusted on the thigh so as to give uniform pressure of 750 pounds. We have found it necessary to tighten the four bolts compressing the springs of the press at intervals throughout the experiment, for otherwise some of the compression is lost during the 7 hour period the press remains in place.

Following release of the press, the leg was gently massaged for a few minutes. The light massage opens up the blood vessels and helps restore the occluded circulation. It is our impression that such massage hastens the onset of shock. Deep anesthesia was maintained for 18 to 24 hours, or until the animal died; during this interval additional pentobarbital sodium was injected intraperitoneally as required. When anesthesia was finally discontinued, the animals which survived were allowed water *ad libitum* and were offered food, which was usually taken.

Blood pressure was determined in the femoral artery by arterial needle puncture (15); hematocrit by means of capillary tubes in an air turbine (16); hemoglobin, Newcomer, and serum protein by the falling drop method (17). The experiments were performed at room temperature varying between 22–24°C. during the 24 hours.

The plasma, gelatin and saline infusions, unless otherwise stated, were given intermittently at definite intervals until the animal had received a total of 40 cc. per kilogram. A total of 6 injections was given spaced at 0, 1, 2, 3, 5 and 8 hours after release of the leg press. A lesser number of infusions, e.g. 4 or 5, differently spaced over a shorter time interval, resulted in fewer survivals.

I. Control series A. This series consisted of 14 deeply anesthetized dogs, the right hind leg of each being subjected to 500 pounds' pressure for a period of 5 hours as recommended by Duncan and Blalock (13). None of the animals received preliminary treatment with morphine, but following release of the press the limb was gently massaged. Of the 14 dogs, 4 or approximately 28 per cent

survived indefinitely, and 10 or 71 per cent died in shock. The average survival period of the dogs that died was 12 hours. The number of animals in this series surviving the procedure was considered too great.

II. Control series B. The preliminary set of experiments convinced us that for uniform production of fatal shock by means of the press it was *a*, necessary to apply pressure in excess of 500 pounds; *b*, to apply the press for a longer time than 5 hours; and *c*, to administer 30 mgm. of morphine in addition to the pentobarbital sodium anesthesia. Therefore in an additional control series consisting of 26 dogs, morphine was administered followed 30 minutes later by intravenous pentobarbital sodium, and 750 pounds' pressure then applied for 7 hours. Following release of the press the limb was lightly massaged. As a result of these procedures only one animal, or 3.8 per cent survived. The average survival period for the remaining 25 animals that died was 11 hours from the time of press removal.

A. *Arterial pressure.* The average mean arterial pressure as determined in the femoral artery of 96 unanesthetized and untrained dogs of all series was 128 mm. Hg. However, within 10 to 15 minutes after administration of morphine subcutaneously the mean pressure fell to an average of 108 mm. Hg. Following intravenous injection of 20 mgm. per kilogram of pentobarbital sodium the mean pressure dropped to an average of 76 mm. Hg. This sharp decline in blood pressure disappeared slowly and by the end of 7 hours the pressure had returned to within an average of 8 mm. Hg of the initial level, or 120 mm. Hg. The effect of the morphine-pentobarbital sodium anesthesia upon blood pressure in representative cases is graphically shown in figure 1. The average figures for all animals are given in table 1.

Pentobarbital sodium alone does not lower arterial pressure, instead it induces an elevation. However, a similar depressing effect of morphine when used in conjunction with ether was reported by Parkins (15). He determined the pressure in the femoral artery by the intra-arterial needle puncture method as was done in these experiments. Green, Nickerson, Lewis and Brofman (18) in a recent study of morphine and barbiturate anesthesia report that the blood pressure (dogs) was lowest when morphine and sodium pentobarbital were used together, but the figures recorded for their six experiments testing the point are higher than ours.

The arterial pressure was near normal levels, i.e., an average of 120 mm. Hg, at the time the press was removed, but thereafter exhibited a slow, steady decline. Six hours following removal of the press the average blood pressure of the 26 control animals was 73 mm. Hg. Shortly before death the pressure fell precipitately. The progressive lowering of blood pressure was accompanied by an increasing pulse rate, marked hemoconcentration, decrease in plasma volume, and gross swelling of the limb due to accumulation of plasma-like fluid in the tissues.

B. *Hemoconcentration.* Blood concentration, as measured by hematocrit and hemoglobin changes does not occur to an appreciable extent during the 7 hour interval the press is applied (table 1). There appeared to be considerable variation among the individuals of the series in this respect, some exhibited slight increases, others decreases in hematocrit and hemoglobin. The average figures

as given in table 1 indicate this variation. Apparently while the press is applied the blood supply is almost completely shut off and little if any flows through the vessels. Following removal of the press and light massage of the limb, hemoconcentration becomes marked. The blood flow through the leg vessels is restored,

TABLE 1

Average blood pressure and blood concentration changes in traumatized dogs given plasma, gelatin and saline infusions

	Number of dogs	Weight	Number survived	Number died	Initial		After morphine		Press removed		After press removal						Survival
											6 hrs.		16–18 hrs.		42–60 hrs.		
					B. P.	Pulse	B. P.	Pulse	B. P.	Pulse	B. P.	Pulse	B. P.	Pulse	B. P.	Pulse	
		kgm.			*mm. Hg*	*min.*	*mm. Hg*	*min.*	*mm. Hg*	*min.*	*mm. Hg*	*min.*	*mm. Hg*	*min.*	*mm. Hg*	*min.*	*hours*
Untreated control	26	16.8	1		140	132	80	108	139	116	74	100					Indef.
		11.6		25	128	97	76	103	121	136	73	172					11
Saline intermittent infusion	23	11.9	8		131	109	79	102	123	152	86	172	75	169	101		Indef.
		11.0		15	128	103	85	112	116	138	70	154	55	150			17
Gelatin intermittent infusion	26	9.4	19		129	113	72	104	114	135	100	155	102	182	105	103	Indef.
		9.8		7	129	106	78	124	113	146	91	170	69	170			16
Gelatin single infusion	11	5.6	1		121	112	66	116	106	116	62	224	81	169			Indef.
		9.4		10	127	88	74	119	117	159	77	127	56	106			17
Plasma intermittent infusion	10	10.9	7		123	107	79	122	135	144	93	198	97	177	113	158	Indef.
		7.9		3	136	110	80	137	130	129	89	128	53	152			14
Average.....		10.6			128	107	76	113	120	137							
					Hematocrit	Hb			Hematocrit	Hb.	Hematocrit	Hb.	Hematocrit	Hb.	Hematocrit	Hb.	
					per cent	*gms. per cent*			*per cent*	*gms. per cent*	*per cent*	*gms. per cent*	*per cent*	*gms. per cent*	*per cent*	*gms. per cent*	
Untreated control	26	16.8	1		46.0	16.7					73.8	26.8					Indef.
		11.6		25	44.4	13.9			42.8	12.6	73.0	23.1					11
Saline intermittent infusion	23	11.9	8		41.3	15.5			42.0	15.3	67.5	20.3					Indef.
		11.0		15	45.9	14.6			44.8	14.1	71.6	21.6					17
Gelatin intermittent infusion	26	9.4	19		46.8	14.5			47.0	14.5	62.0	19.8	57.4		52.1		Indef.
		9.8		7	45.3	13.2			48.5	14.7	65.3	21.2					16
Gelatin single infusion	11	5.6	1		48.2	15.1			51.1	15.5	70.2	23.4	62.6	20.5			Indef.
		9.4		10	45.9	15.3			49.1	16.3	69.2	22.8	71.0	24.9			17
Plasma intermittent infusion	10	10.9	7		44.3	13.7	41.8	12.6	43.9	13.7	58.5	18.4	52.0	16.4	43.4	14.0	Indef.
		7.9		3	53.1	16.9	49.6	15.9	45.6	13.8	66.2	20.8	61.4	20.7			14

and the plasma apparently passes freely through the capillaries damaged by 7 hours of anoxia.

The greatly increased hematocrit and hemoglobin concentrations which invariably occur and attain their peak 6 to 8 hours after releasing the press, indicate that the decrease in the volume of circulating fluid is probably due chiefly, if not

entirely, to loss of plasma into the area of injury. This is also shown by the autopsy findings. The degree of hemoconcentration observed is strikingly similar to that noted after release of leg tourniquets. The hematocrit increased 71 per cent 6 to 8 hours after removing the press, while the hemoglobin increased 83 per cent.

C. *Plasma volume reduction.* Plasma volume studies were made on 7 dogs of the control series. The blue dye T-1824 was used according to the technique described by Gregersen and Stewart (19). The average normal plasma volume of the seven dogs was 53 cc. per kilogram body weight. Six to 11 hours following release of the press, the plasma volume had declined to an average of 27.5 cc. per kilogram thus representing approximately a 48 per cent reduction (fig. 1). Presumably most, if not all, of the plasma was lost into the tissues of the injured leg, at any rate there was nothing to indicate a generalized transudation. At autopsy when the tissues of the swollen limb were cut, clear, straw colored fluid dripped from the cut surfaces. Other tissues examined were relatively dry and appeared dehydrated upon gross examination.

D. *Autopsy findings.* In general, the gross pathological findings observed in the animals were similar to those occurring after release of leg tourniquets. There was some congestion of the duodenum and jejunum, and an occasional dog exhibited hemorrhage into the lumen of the gut. Some congestion of the liver, kidneys and spleen was noted. Macroscopically the adrenal cortex showed a few hemorrhagic areas. Only one of the dogs of the control series survived long enough to develop infection, but infection with Cl. welchii or other gas producing organism was observed in the injured area of two gelatin infused dogs that survived the procedure. Oliguria and hematuria were characteristic findings in most of the animals following release of the press.

The limb was swollen at death, and the swelling apparently represented accumulations of plasma-like fluid which could be readily expressed from a cut surface. After death, in both gelatin or salt infused animals, fluid dripped freely from any cut made into the thigh tissue. The subcutaneous areas of the entire leg were distended with fluid, and occasionally the fluid extended into the subcutaneous areas of the flank and of the lower abdomen.

In those animals which recovered as a result of either saline or gelatin treatment, the leg remained swollen for a week or more and was paralyzed. Eventually necrosis appeared at the site of the greatest pressure, but despite the skin necrosis and sloughing, the dogs had normal appetites and seemed healthy and vigorous. The animals were usually kept for ten days in the laboratory and then sacrificed. The paralysis persisted throughout this interval.

III. Gelatin infusion series. Twenty-six anesthetized animals were used in the experiments. They received treatment similar to series B of the control group until the press was released at the end of 7 hours. Intravenous gelatin infusions were then started. Each dog received a total of 40 cc. per kilogram given intermittently as 6 injections of 6.6 cc. per kilogram each over 8 hours. The animals surviving at the end of 24 hours when anesthesia was discontinued were offered

water and food. They exhibited thirst at this time and eagerly took large quantities of water.

Nineteen, or 73 per cent, of the 26 gelatin infused dogs did not develop shock and survived indefinitely, and 7 or 27 per cent died, with an average survival period of 16 hours. The average life-span of the gelatin infused animals that died following press removal was longer than that of untreated controls (table 1).

It is evident from the data that gelatin infusions given intermittently over 8 hours after releasing the press are efficacious in preventing shock induced by this procedure. Duncan and Blalock (14) in a series consisting of 15 anesthetized dogs subjected to the press, administered a single massive transfusion of plasma equivalent to 3.3 per cent of the body weight over a two hour period starting immediately after removing the press. Six, or 40 per cent, of the dogs lived, and 9, or 60 per cent, died. Apparently gelatin infusions if given intermittently are a better prophylactic treatment for preventing the shock resulting from the press than is a single large transfusion of plasma.

Attempts to prevent shock by administering the gelatin in 4 or 6 injections over 5 to 7 hours proved less satisfactory than 6 injections over a longer interval. Only a few animals survived when 3 to 4 hours were permitted to lapse between release of the press and the first gelatin injection; by this time hemoconcentration was well established and the arterial pressure lowered. Thus it seems reasonably certain that 6 gelatin infusions of 6.6 cc. per kilogram each, given over 8 hours, represent the minimum which will result in a good number of survivals.

The average initial arterial pressure of the gelatin infused dogs was 129 mm. Hg. Six hours after press release the average pressure for the group was 100 mm. Hg in the 19 survivors, and 91 mm. Hg in the group of 7 which died later. Eighteen hours after the press was removed the pressure was 102 mm. Hg in the surviving group. One dog had a pressure of 59 mm. Hg at 18 hours but eventually recovered when permitted to have water. Of the 7 animals that died, only a few survived for more than 16 hours.

Hemoconcentration although present was less marked in the gelatin infused dogs as compared to untreated controls. For example, six hours after releasing the press, the average hematocrit and hemoglobin figures for the control series were 73 per cent and 23.1 grams per cent respectively. The average reading for the hematocrit of the gelatin infused dogs which survived was 62 per cent and the average for hemoglobin was 19.8 grams per cent. Complete return to normal values seldom occurred before 42 to 65 hours (table 1, fig. 1).

IV. Saline infusion series. Since the gelatin was a 5 per cent solution in physiological saline, it was imperative that the experiments be controlled by identical studies in which isotonic salt solution was used as the injection fluid. Several investigators studying the comparative value of plasma substitutes have reported that salt solution *per se* is effective in a certain percentage of cases of shock induced by various means (7, 11, 20, 21). Recently the writers observed that intermittent infusions of saline representing a total of 33 cc. per kilogram administered in 5 injections over a 7 hour period, effectively prevented the shock resulting from leg muscle trauma in approximately 50 per cent of the cases tested.

It is true that similar infusions of gelatin likewise prevented shock in 50 per cent of the traumatized dogs, but it was obvious that in so far as this particular type of shock is concerned, the gelatin was no more effective as a plasma substitute than the saline in which it was dissolved.

Twenty-three anesthetized dogs were used in the experiments for testing the effectiveness of salt solution. A total of 40 cc. per kilogram was given in 6 injec-

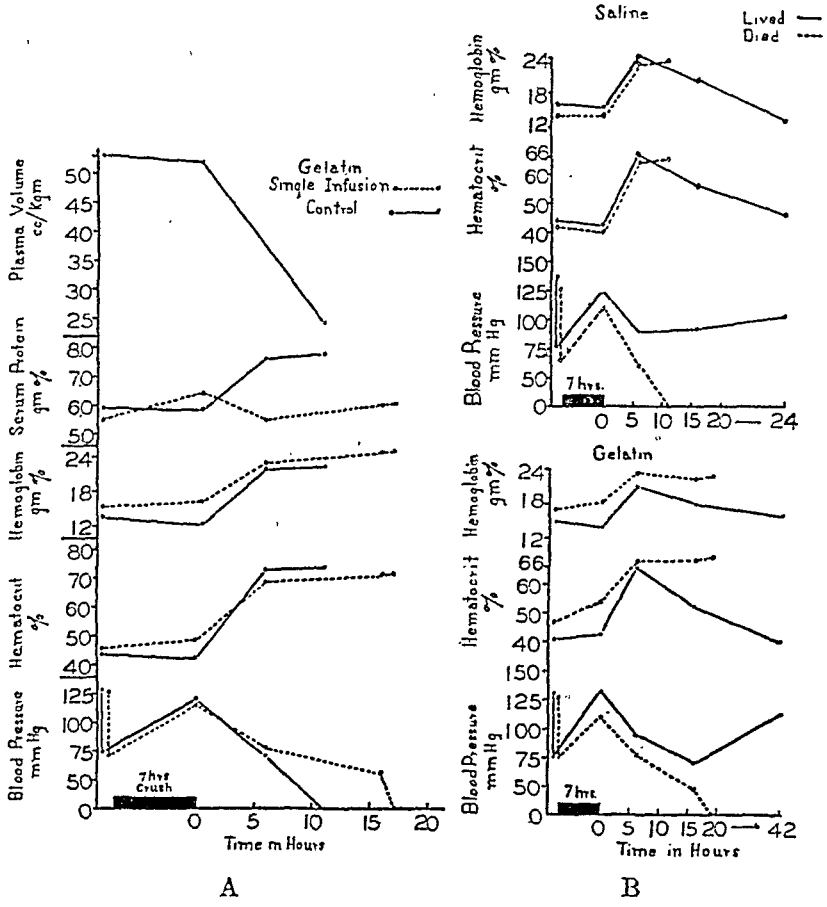

Fig. 1A. Blood pressure and hemoconcentration changes exhibited by typical cases of *a*, the control series, and *b*, the series which received a single massive infusion of gelatin. The plasma volume changes represent the average decline shown by 7 untreated controls.

Fig. 1B. Blood pressure and hemoconcentration changes shown by representative cases of the gelatin and saline infusion series. The low blood pressure during the press interval is an effect of the morphine-pentobarbital sodium anesthesia.

tions of 6.6 cc. per kilogram each over an 8 hour interval as in the gelatin studies. Of the 23 dogs treated, 15 or 65 per cent died with an average survival of 17 hours from the time the press was removed, and 8 or 35 per cent survived indefinitely (table 1, fig. 2).

If the saline treated series is compared with that receiving gelatin the greater survival value of the gelatin becomes evident. Thus of 26 animals receiving gelatin, 19 or 73 per cent lived and 27 per cent died. The data indicate therefore that gelatin is definitely more effective in preventing shock induced by the leg

press, than is saline in equal quantity given intermittently over the same time interval. However, the intermittent saline infusions are of definite value as a prophylactic in preventing this type of shock. This is shown by the number of animals surviving (8 of 23) when given saline, as compared to the one survivor among the 26 animals of the untreated control series.

V. Single massive infusion of gelatin. All infusions in the previous series of experiments were of the intermittent type. In this study the gelatin was given as a single infusion equivalent to 40 cc. per kilogram. The injection was begun within a few minutes after release of the leg press and before the arterial pressure had declined or hemoconcentration become evident. The gelatin was injected into the jugular vein at a rate not exceeding 1 cc. per kilogram per minute.

Eleven dogs were used and but one survived. The average life-span after infusion was 17 hours, an interval six hours longer than that of the untreated series. The injured leg of all animals of the group exhibited marked swelling at the end of the infusion and the ultimate size attained by the limb was considerably greater than observed in any untreated control. Apparently, the gelatin when administered as a single massive infusion, rapidly passes through the injured capillaries into the tissues. Several students of shock have reported similar observations after using large infusions of saline or plasma. At the end of the 6th hour after the single gelatin infusion, the hemoconcentration as judged by hematocrit and hemoglobin determinations was as severe as in untreated controls (table 1). At this time the average arterial pressure of the 10 animals comprising the group which died was 77 mm. Hg, the hematocrit 69.2 per cent, and hemoglobin 22.8 grams per cent (fig. 1 and table 1).

Since fluid containing both 5 per cent gelatin and 0.9 per cent saline was ineffective in preventing shock when given as a single infusion, it was considered unnecessary to test the effect of single massive injections of physiological saline.

The experiment demonstrates that small intermittent infusions of gelatin, spread over an 8 hour period following trauma are more effective in preventing shock than equal quantities of gelatin administered as a single massive injection. This was found to be true also for plasma transfusions as shown in earlier work on shock induced by release of tourniquets.

VI. Intermittent transfusion of plasma. For comparison with the dogs receiving intermittent infusions of gelatin and saline, ten animals subjected to the press were given similar infusions of pooled, heparinized dog plasma. The total amount administered was 40 cc. per kilogram given in 6 injections over 8 hours. Three of the 10 plasma infused dogs or 30 per cent died, and 7 or 70 per cent survived. The experiment failed to demonstrate any marked superiority of plasma over gelatin in preventing shock from the procedure employed, when both were given intermittently. The pertinent data are given in table 1.

SUMMARY

1. The application of a Duncan-Blalock press to one hind leg of the deeply anesthetized dog for 7 hours at 750 pounds' pressure, induced fatal shock in 25 of 26 untreated control animals. The average survival period following release of

the press was 11 hours. Anesthesia was continued for 18 to 24 hours in all of the experiments or until the animal died.

2. The shock is characterized by intense hemoconcentration, progressive fall in arterial pressure and loss of plasma. There is an average decline in plasma volume of 48 per cent. The greatly swollen leg indicated that most if not all of the plasma volume decline could be accounted for by plasma loss into the injured area.

3. A single massive intravenous infusion of gelatin representing 40 cc. per kilogram, given immediately after removing the leg press, failed to prevent fatal shock in 10 of 11 dogs. The gelatin was apparently not retained in the circulation but rapidly passed through the injured capillaries of the limb.

4. Six intermittent gelatin infusions of 6.6 cc. per kilogram each, administered over an 8 hour interval from the time of removing the leg press, prevented shock in 19 or 73 per cent of 26 animals.

5. Intermittent infusions of 0.9 per cent saline, administered in equal dosage and over the same time interval as the gelatin, proved to be less effective. Eight or 35 per cent of 23 dogs survived, whereas 15 or 65 per cent died.

6. Small intermittent infusions of pooled, heparinized dog plasma administered in the same dosage and over the same time interval proved no more effective in preventing shock than similar infusions of gelatin.

7. Intermittent infusions of plasma or plasma substitutes, when given in small amounts over a period of hours, are more effective in preventing shock than is a single massive infusion.

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THE MODE OF ACTION OF AN ANTICOAGULANT DERIVED FROM TISSUES[1]

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Received for publication January 29, 1944

Sphingomyelin has been demonstrated to be responsible for the specific action of the phosphatide fraction of the anticoagulant tissue albumin complex (1). In its presence the coagulation of citrated, recalcified plasma is prolonged or inhibited. The rate of coagulation, according to Quick (2), is directly proportional to the concentration of available thrombin. Thrombin titration experiments show that anticoagulant tissue albumin reduces the amount of available thrombin. Such reduction may be effected by inhibition of the formation of thrombin in the first phase or by its inactivation before it comes into contact with fibrinogen in the second phase of coagulation. The experiments to be reported show that the lipid anticoagulant interferes with thromboplastic activity and that it neutralizes thrombin in isolated systems. Its anticoagulant activity is selectively inhibited by Reinecke salt. The finding that the coagulation of normal recalcified plasma is accelerated by Reinecke salt suggests that sphingomyelins, normally present in blood, may play a similar rôle in the prevention of coagulation.

METHODS AND REAGENTS. The test employed for coagulation time and the preparation of tissue extracts, anticoagulant tissue albumins and anticoagulant sphingomyelins have been described in a previous communication (1).

Fibrinogen solution was prepared from citrated dog plasma by the Florkin method (3). The fibrinogen was precipitated at least three times with NaCl at 55 per cent saturation. Test solutions of fibrinogen were made with concentrations of fibrinogen adjusted to 0.5 per cent and of NaCl to 0.9 per cent.

For preparation of thrombin solutions Eagle's method (4) was utilized. The standard, full strength, thrombin solutions were brought to the volume of plasma from which they were isolated. The yield in thrombin by this method is about 65 per cent of the theoretical (5). Testicular extract (6) served as thromboplastin. Heparin (Lederle), protamine sulfate (Squibb and Sons), Reinecke salt (Eastman-Kodak) were the commercial preparations employed.

I. *Rôle of the Lipid Anticoagulant in the First Phase of Coagulation. Calcium.* Experiments were designed to determine the possible influence of tissue anticoagulant on calcium. When citrated plasma, recalcified with optimal quantities of calcium did not coagulate in the presence of adequate amounts of tissue anticoagulant, even an excess of calcium had no effect. This indicated that the action of tissue anticoagulant is not dependent upon calcium ions.

[1] Aided by grants from the John and Mary R. Markle Foundation.

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Platelets. It was attempted to ascertain the influence of the anticoagulant tissue albumin on the disintegration of platelets. For this purpose smear preparations (7) were used of citrated, recalcified plasma mixtures on slides coated with brilliant cresyl-blue. No striking difference was found in the behavior of the platelets due to the presence of the lipid anticoagulant. Small clusters of agglutinated platelets were found both in the presence and in the absence of the

TABLE 1

Effect of an excess of thromboplastin on the activity of tissue anticoagulant

Solution of tissue anticoagulant in ml*		0.5		0.1	0.2	0.3	0.4	0.5
Solution of thromboplastin in ml.			0.2	0.2	0.2	0.2	0.2	0.2
Saline in ml.	0.5		0.3	0.2	0.1			
Clotting time of plasma in sec.†	348	>1800	9	9	9	9	9	9

* Tissue anticoagulant was isolated from spleen and its solution had a protein concentration of 1.56 per cent.

† One-half milliliter of citrated plasma, recalcified with 0.3 ml. of a 1.1 per cent $CaCl_2$ solution, was the test-mixture.

TABLE 2

Effect of an excess of tissue anticoagulant on the activity of thromboplastin

ANTICOAGULANT EXTRACT OF SPLEEN*	COAGULATION TIME OF PLASMA†
ml.	*sec.*
0.00	310
0.05	700
0.10	1140
0.25	2450 loose net of fibrin only
0.50	fluid in more than 14 hrs.

Effect of isolated protein fractions of the above extract on the coagulation time of plasma

PROTEIN FRACTIONS	COAGULATION TIME OF PLASMA
	sec.
0.2 ml. of solution of globulins	106
0.2 ml. of solution of albumins	>3600

* Extract of spleen was prepared from pig spleens and had a protein concentration of 2.56 per cent. Globulins and albumins were separated by salting out precipitation with ammonium sulfate. The dialysed solutions of globulins had a protein concentration of 0.96 per cent, that of albumins 1.48 per cent.

† Test solutions were made of 0.5 ml. of citrated plasma and 0.3 ml. of a 1.1 per cent $CaCl_2$ solution.

anticoagulant within a minute after recalcification. Shortly before the controls coagulated, in six minutes, the platelets seemed to undergo disintegration in both series. The plasmas containing the anticoagulant remained fluid for more than 30 minutes.

Thromboplastin. Tables 1 and 2 show that the coagulation time of recalcified plasma may be altered at will by the addition of varying amounts of thrombo-

plastin and of anticoagulant tissue albumin. The rate of coagulation is dependent on the ratio of the concentration of these two substances.

When thromboplastin is in such excess that the coagulation time corresponds to the prothrombin clotting time of Quick (8) the activity of the anticoagulant tissue albumin is annulled. It should be noted in table 1 that the prothrombin clotting time is uninfluenced even when tissue anticoagulant is added to plasma in amounts that prevent coagulation for over 30 minutes in the absence of added thromboplastin.

When anticoagulant albumins are in excess the activity of thromboplastic globulins is inhibited. As shown in table 2 increasing amounts of an anticoagulant extract of spleen progressively prolong the coagulation time of standard plasma mixtures. In the whole extract the activity of thromboplastic globulins is inhibited by anticoagulant albumins. On isolation, however, their antagonistic activity becomes apparent.

The antagonistic activity of thromboplastic globulins and anticoagulant albumins is demonstrable by testing inert mixtures of these two substances. Addition of such inert mixtures or of similar combinations of kephalins and sphingomyelins to citrated plasma does not influence the coagulation time. Similarly, dilution of these inert mixtures does not affect the rate of coagulation.

TABLE 3

Effect of incubating 0.2 ml. of thrombin (⅓ strength) with 0.3 ml. of tissue anticoagulant and saline, respectively, at 37° prior to the addition of 0.5 ml. of fibrinogen solution

TIME OF INCUBATION	WITH SALINE	WITH TISSUE ANTICOAGULANT
minutes	*coagulation time in seconds*	
0	25	34
5	25	35
10	26	33
30	24	34

II. *Rôle of the Lipid Anticoagulant in the Second Phase of Coagulation. Thrombin.* Anticoagulant tissue albumin added to thrombin retards the coagulation of fibrinogen. Table 3 shows that the interaction between tissue albumin and thrombin is independent of the period of incubation. This immediate loss in thrombin activity on addition of tissue albumin contrasts with the gradual, progressive loss that results in the presence of fresh serum albumin under similar conditions (9).

As seen in table 4 the coagulation time of isolated fibrinogen-thrombin systems varies with the concentration of sphingomyelin, the active component of the tissue albumin complex. The prompt inhibition of thrombin is independent of the time of incubation. The amount of thrombin inactivated does not increase in strict stoichiometric relation with the amount of sphingomyelin. The time interval from the beginning of transformation of fibrinogen to fibrin and complete gelification of fibrinogen grows as the concentration of sphingomyelin is

increased. The graded thrombin inactivation may be imitated by progressive dilution of the thrombin preparation with saline.

As shown in table 5, 0.8 mgm. of anticoagulant sphingomyelin inhibited coagulation of recalcified, citrated plasma for over 30 minutes. The same amount of sphingomyelin only delayed but did not prevent the transformation of fibrinogen to fibrin in the presence of less than half the thrombin formed from ½ ml. of plasma during a regular clotting process. Larger quantities of the lipid anticoagulant, 1.0 to 5.0 mgm., likewise delayed but did not completely prevent transformation of fibrinogen to fibrin. To obtain prolongation of fibrin formation comparable to that effected by 2.5 mgm. of the lipid anticoagulant, 300 fold

TABLE 4

Effect of sphingomyelin on action of thrombin

Increasing amounts of sphingomyelin, dispersed in saline, are mixed with 0.5 ml. of fibrinogen solution immediately before addition of 0.2 ml. of thrombin solution (full strength). Controls with saline dilutions. Coagulation times in seconds.

SALINE ALONE		PURIFIED SPHINGOMYELIN IN SALINE	
0.0 ml	12–13	0.0 mgm. in 0.0 ml. of saline	12–13
0.05 ml	12–14	0.1 mgm. in 0.05 ml. of saline	13–15
0.1 ml	12–14	0.2 mgm. in 0.1 ml. of saline	14–17
0.2 ml	13–14	0.4 mgm. in 0.2 ml. of saline	17–18
0.3 ml	11–12	0.6 mgm. in 0.3 ml. of saline	22–23
0.4 ml	11	0.8 mgm. in 0.4 ml. of saline	34–41
0.5 ml	10	1.0 mgm. in 0.5 ml. of saline	50–80

TABLE 5

Effect of 0.8 mgm. of purified sphingomyelin on the coagulation time of*

0.3 ML. OF THROMBIN (FULL STRENGTH)† 0.5 ML. OF SOLUTION OF FIBRINOGEN		0.5 ML. OF CITRATED PLASMA 0.3 ML. OF 1.1% $CaCl_2$ SOLUTION	
Saline alone	Sphingomyelin in saline	Saline alone	Sphingomyelin in saline
sec.	*sec.*	*sec.*	*sec.*
10	34–41	210	>1800

* Sphingomyelin was dissolved in 0.4 ml. of saline.

† Thrombin was prepared from the plasma used in the recalcification experiment.

dilution with saline of the thrombin solution was necessary. These and similar observations indicate that tissue anticoagulant is more effective in preventing formation of thrombin than in inhibiting the action of formed thrombin.

Fibrinogen. Purified fibrinogen, incubated with the anticoagulant, clots readily upon the addition of an excess of thrombin. This may indicate that the fibrinogen-thrombin interaction remains unaffected.

The Selective Inhibition of the Anticoagulant Activity of Sphingomyelin. Protamine. Chargaff (10) offered evidence that heparin, synthetic polysulfuric acids and sulfurated lipid anticoagulants are inactivated by protamine. No concentration of protamine from 0.0025 mgm. in 1 ml. of recalcified plasma to quantities

that precipitated fibrinogen (11) effected complete inhibition of the anticoagulant action of tissue albumins. This may be due to the fact that sphingomyelins do not form insoluble complexes with protamine (12) and that the sphingomyelins used as anticoagulants do not contain sulfur (1).

Reinecke salt. It is well known that Reinecke salt (ammonium-tetrathiocyano-diammonochromate) in methanolic solutions precipitates sphingomyelin as insoluble sphingomyelin-Reineckate (13, 14). This complex, it was postulated, would be inactive as an anticoagulant in the analogy of the protamine-heparin combination (10), and so it proved to be.

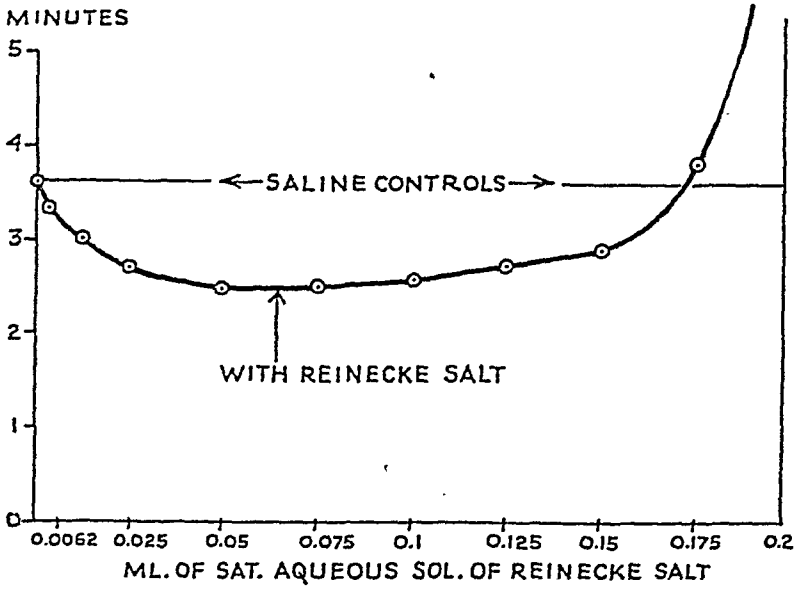

Fig. 1. Abscissa: Reinecke salt, in milliliter of its saturated aqueous solution, added to citrated plasma.

Ordinate: Coagulation time of recalcified plasma in minutes. One-half milliliter of citrated plasma was mixed with Reinecke salt prior to recalcification with 0.3 ml. of a 1.1 per cent $CaCl_2$ solution. Uniform total volume of the coagulating mixtures was maintained by the addition of saline.

Reinecke salt was found to influence the coagulation time of normal recalcified plasma: Less than 0.1 ml. of a saturated aqueous solution of Reinecke salt usually did not evoke any visible precipitation when added to 0.5 ml. of citrated plasma. The plasma only took up the pink-red color of the salt. No other change was noted even when the plasma was left in contact with the salt for a number of hours. Upon recalcification, however, coagulation followed at an accelerated rate. Figure 1 shows that the acceleration may reach a maximum with less than 0.1 ml. but it should be noted that the quantity of the salt required for maximal action varies with each individual plasma sample. More than 0.1 ml. of a saturated aqueous solution of Reinecke salt added to the same quantity of citrated plasma usually evoked immediate turbidity and soon afterwards a loosely woven fibrin net separated. Upon recalcification, coagulation did not always follow.

When it did occur, it was accelerated, but even then it was prone to be focal with balls of fibrin suspended in the liquid matrix. Usually coagulation did not follow recalcification when more than 0.2 ml. of the salt was added to 0.5 ml. of plasma.

Reinecke salt alone did not interfere with the action of thrombin for citrated plasmas containing the salt in concentrations that did not cause precipitation coagulated readily upon addition of thrombin. This is shown in table 6. It should be noted that coagulation was effected by a weak thrombin solution in the same length of time irrespective of the presence of Reinecke salt in the plasma.

When sphingomyelin and Reinecke salt were brought together in aqueous solution the red color of the salt changed immediately to pink without visible

TABLE 6

Ineffectiveness of Reinecke salt on the action of thrombin

Reinecke salt in ml. of a saturated saline solution		0.025	0.05	0.075	0.1	0.15
Saline in ml.	0.1	0.05	0.05			
Coagulation time in seconds*	40	40	40	40	40	40

* The coagulating systems consist of 0.5 ml. of citrated plasma and of 0.2 ml. of thrombin solution ($\frac{1}{3}$ strength).

TABLE 7

Effect of Reinecke salt on the activity of the tissue anticoagulant

Solution of a purified tissue anticoagulant (prot. conc. 0.52%) in ml.		0.1	0.1	0.1	0.2	0.2	0.2
Reinecke salt in ml. of its saturated saline solution			0.025	0.05		0.05	0.1
Saline	0.2	0.1	0.15	0.1			
Coagulation time* in seconds	165	>1800	215	160	>1800	182	155

* The coagulating systems consist of 0.5 ml. of normal, citrated plasma and 0.5 ml. of a 1.1 per cent $CaCl_2$ solution.

precipitation. At the same time sphingomyelin lost its anticoagulant activity. As is shown in table 7 when 0.1 or 0.2 ml. of an anticoagulant tissue albumin solution was added to a rapidly coagulable plasma, on recalcification coagulation did not occur within 30 minutes. The addition of suitable amounts of an aqueous solution of Reinecke salt successfully counteracted the anticoagulant action of tissue albumin. It was found in further experiments that when the salt was added to citrated plasma containing the tissue anticoagulant in suitable concentration prior to recalcification, coagulation took place in the same manner and time as it did in similar combinations without the tissue anticoagulant. However, when Reinecke salt was added to recalcified plasma that had not coagulated due to the presence of the tissue anticoagulant or its lipid component, coagulation only took place after a lag period. The regular occurrence of such

lag period indicated that the presence of the anticoagulant interfered with the formation of thrombin until Reinecke salt was added.

Discussion. The phospholipid sphingomyelin is a normal constituent of plasma. Its concentration was estimated by Thannhauser (13) to be 15 to 35 mgm. per cent and by Ramsay and Stewart (14) to be as high as 56 to 70 mgm. per cent. The facts that sphingomyelin isolated from animal tissues is anticoagulant and that Reinecke salt, an inhibitor of the anticoagulant action of sphingomyelins, accelerates the rate of coagulation of normal plasma suggest that the sphingomyelins of the blood may act similarly. In the fluid blood sphingomyelin or a related substance may play the rôle of a natural thrombin inhibitor and a natural antithromboplastin. It is suggested that the removal of this inhibitor in the first phase of coagulation by thromboplastin and Ca is necessary to activate the coagulating mechanism.

It is not a new suggestion that the inactive thrombin precursor, prothrombin, is a combination of thrombin plus an inhibitor. A number of investigators (e.g., 15, 16) have suggested that thrombin is freed by thromboplastin and Ca from hypothetical inhibitors. Sphingomyelin may be such an inhibitor. Whether it acts directly upon thrombin or upon a proteolytic proenzyme, a prerequisite for thrombin activation (17, 18, 19) can only be hypothesized at present. If sphingomyelin inhibits a proteolytic proenzyme it would be a specific example of the more general phenomenon of inhibition of proteolytic enzymes by higher unsaturated lipids (20).

Added thromboplastin accelerates coagulation by speeding up the rate of thrombin formation (17). Added tissue anticoagulant or its lipid component retards or prevents coagulation by slowing down the rate of thrombin formation. When both are added to a coagulating mixture, the coagulation time is a function of the relative concentration of the two. These facts suggest that both may have the same locus of action. How the antithromboplastic action is exerted is not known. It is possible that an inactive kephalin-Ca-sphingomyelin combination results. In the normal process of thrombin activation thromboplastin is actually consumed (21). This fact may imply that during the normal coagulation process part of the thromboplastin is utilized for the inactivation of plasma sphingomyelin. Accordingly, combination of an excess of added sphingomyelin with available thromboplastin through Ca would leave the normal sphingomyelin of plasma available for the inhibition of thrombin.

SUMMARY

1. The addition of increasing quantities of sphingomyelin or of a sphingomyelin-protein complex to citrated, recalcified plasma progressively prolongs coagulation time. An excess of thromboplastin annuls this anticoagulant action. The coagulation time of standard plasma mixture to which both lipid anticoagulant as well as thromboplastin have been added is dependent on the ratio of the concentrations of these two substances. Inert mixtures of the two maintain their equilibrium during the entire coagulation process. These experiments indicate that tissue anticoagulant and thromboplastin are antagonists.

2. Anticoagulant tissue albumin or sphingomyelin inactivates isolated thrombin. The inactivation of thrombin is instantaneous and the effect does not increase on incubation. The amount of thrombin inactivated varies directly with the amount of tissue anticoagulant employed. These experiments indicate that tissue anticoagulant is an antithrombin.

3. Reinecke salt selectively inhibits the anticoagulant action of sphingomyelin. The inhibition is referable to a complex formation. Suitable concentrations of Reinecke salt invariably accelerate the coagulation rate of normal recalcified plasma.

4. Evidence is presented in support of the assumption that the sphingomyelin of the blood may be a natural antithromboplastin and thrombin inhibitor. Its removal or inactivation by thromboplastin and Ca may be the necessary first step for the activation of thrombin.

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COMPARISON OF THE NUTRITIVE VALUE OF DEXTROSE AND CASEIN AND OF THE EFFECTS PRODUCED ON THEIR UTILIZATION BY THIAMINE[1]

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Received for publication February 4, 1944

Our knowledge of the nutritive value of the individual proteins, fats and carbohydrates is limited largely to observations made on natural or synthetic diets which contain varying proportions of these three main foodstuffs, and many minerals and vitamins. Under such complicated circumstances it becomes very difficult to compare the nutritive value of the individual representatives of the three main foodstuffs or the effects produced by the different vitamins on their utilization.

Such comparisons can be made, however, when the experiments are so arranged that one constantly available foodstuff, a purified carbohydrate, fat or protein, constitutes the sole source of nourishment (1, 2). In such "single food choice" experiments the animals must all start at approximately the same age and weight and must previously have been kept on essentially the same diet. The length of time they live on the single food and the amount of activity that they show, provide quantitative measures of the nutritive value of the foodstuff under examination.

In previous experiments the nutritive value of dextrose was studied in rats, using this single food choice method. Given no food rats lived only 4 days, while with free access to a purified dextrose they lived on the average 37 days, that is, over nine times as long as on no food at all. Furthermore, the rats given dextrose remained very active for the first 20 to 25 days. When given access to a 0.02 per cent solution of thiamine chloride the rats on dextrose drank it freely, and as a consequence lived much longer than on dextrose alone. Their survival times averaged 76 days, almost twenty times as long as on no food at all and over twice as long as on dextrose alone. They remained very active for as long as 60 days after the start of the diet. Thus, under the simplest conditions these experiments demonstrated the great effect which thiamine has on the utilization of the carbohydrate dextrose.

A comparison has now been made, using this method, of the nutritive value of dextrose, one of the outstanding carbohydrates, with that of casein, one of the outstanding proteins.

METHODS. The activity cages used for these experiments have been described in detail in previous communications (2, 3). Each cage consisted of a revolving drum, 32 cm. in diameter, a cyclometer, and a living compartment which contained a non-spillable food cup and places for two graduated inverted water bottles. The large meshed bottom of the living compartment made it possible

[1] Supported by a grant from the Corn Industries Research Foundation, New York City.

for all the feces to drop out of range. In the drums the feces also dropped out of reach. Thus the arrangement reduced coprophagy to a negligible minimum.

Each rat was placed in an individual cage during a control period when it was approximately 45 days of age and kept on our stock diet. After 15 to 20 days, when the body weight had increased to a level between 119 to 149 grams, the single food diet replaced the stock diet. However, only those rats were used which in the 15 to 20 day period on the stock diet had shown normal growth curves, regular 4 to 5 day estrous cycles and general good health.

Group I (21 rats) was placed on dextrose and group II (16 rats) on casein (fat and water soluble vitamin-free). Group III (12 rats) and group IV (14 rats) were placed on dextrose and casein respectively and were given constant access to a 0.02 per cent solution of thiamine hydrochloride.

Daily records were made of activity (number of revolutions of revolving drum), food and water intake, and vaginal smears; and weekly records were made of body weight. The rats were inspected at least once weekly for signs of nutritional deficiency.

Single food choice. Survival times. Table 1 summarizes the results. The 16 rats on casein lived an average time of 33 days, which fell short only 4 days of that of the 37 day survival period of the 21 rats on dextrose. The survival times of both groups far surpassed that of the control group of 11 rats on no food at all (4 days).

When given access to the thiamine chloride solution the 14 rats of group IV on casein lived 55 days or 66.7 per cent longer than on casein alone; while the 12 rats of group III on dextrose lived 73 days or 97.3 per cent longer than on dextrose alone.

Body weight. Figure 1A summarizes the results produced on body weight of the four groups of rats by the different single food diets. The ordinates show body weight in grams; the abscissae time in days. It will be seen that the rats on casein and dextrose lost weight at almost the same rate during the entire 40 day period.

When given access to the thiamine solution the rats of groups III and IV also lost weight at almost the same rate. After the first 10-day period the rate of weight loss was slower than that of the rats not receiving thiamine. Thus the effects of thiamine did not appear before the second 10-day period.

Activity. Figure 1B summarizes the results. The ordinates show activity in number of revolutions of the revolving drum; the abscissae time in days. Both groups of rats on casein became less active immediately after starting on the single food diet. Thiamine had practically no effect on their activity. In marked contrast both groups of rats on dextrose became definitely more active. The rats of group I remained very active for the first twenty days, then showed a sharp decrease, while the rats of group III maintained a high level of activity for 60 days.

Food intake. Figure 2A summarizes the results. The ordinates show food intake in grams; the abscissae time in days. The two groups of rats each on dextrose and on casein showed marked differences in food intake. For the first

few days on the single food diet both groups of rats on dextrose ate almost as much of the dextrose as they had previously eaten of the stock diet. During the first 10-day period their average daily food intake dropped only from 13 to 10 grams. During the following 10-day periods the intake of the rats on dextrose alone decreased at a steady rapid rate, while that of the rats on thiamine decreased at a much slower but equally constant rate. In marked contrast the two groups of rats on casein showed a sharp drop in food intake at once after starting on the single food diet. Some rats did not eat any of the casein on the first and second days. The others ate 2 to 5 grams. For the first 10-day period the intake of both casein-fed groups remained the same. Later the intake of the rats on casein alone decreased at a slow rate, while the intake of the rats on

TABLE 1

GROUP	DIET	NUMBER OF RATS	AVERAGE AGE AT START	AVERAGE WEIGHT AT START	SURVIVAL TIME	AVERAGE SURVIVAL TIME
			days	*grams*	*days*	*days*
I	Dextrose (anhydrous)	21	64 (56–71)	137 (120–149)	28, 29, 32, 33, 33, 34, 35, 36, 36, 36, 37, 37, 38, 39, 39, 40, 40, 41, 42, 42, 54	37
II	Casein (Labco)	16	63 (55–69)	132 (124–145)	26, 26, 26, 27, 28, 28, 32, 32, 32, 33, 33, 34, 39, 39, 45, 46	33
III	Dextrose (anhydrous) plus Vitamin B_1 (0.02%)	12	62 (58–66)	139 (130–148)	62, 64, 65, 67, 72, 73, 74, 75, 76, 76, 87, 87	73
IV	Casein (Labco) plus Vitamin B_1 (0.02%)	14	60 (53–63)	137 (119–149)	36, 40, 41, 42, 42, 52, 55, 56, 60, 64, 65, 68, 70, 72	55
	No food	11	57 (40–72)	141 (118–210)	3, 4, 4, 4, 4, 4, 4, 4, 4, 5, 6	4

thiamine remained on the same level. The rats of this group continued to eat about 3.5 grams per day up to the last day before they died.

Water intake. Figure 2B summarizes the results. The ordinates give total fluid intake (in groups III and IV—including water from thiamine solution); the abscissae time in days. The dextrose and casein groups of rats showed very different intake curves. The water intake curves of the two groups of rats on dextrose closely paralleled one another. They both showed a marked decrease from daily averages of approximately 24 cc. for the last 10 days on the stock diet to 12 cc. for the first 10 days on the single food diets. During the following 10-day periods the water intake at first decreased less rapidly, then tapered off to a flat level at 5.5 cc. In marked contrast the rats of the two groups on casein drank slightly more during the first 10 days on the single food diet than during

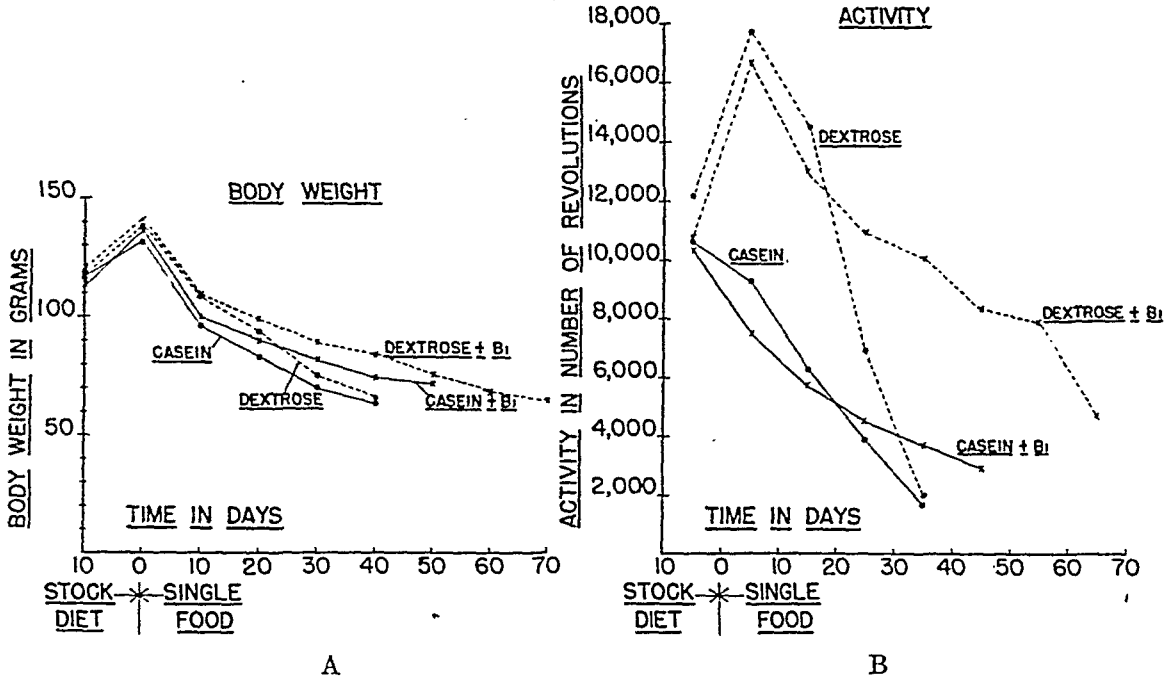

Fig. 1A. Graph showing body weight curves of rats on single food choice diets.

Group I—dextrose (21 rats). Group II—casein (16 rats). Group III—dextrose + B_1 (12 rats). Group IV—casein + B_1 (14 rats).

Fig. 1B. Graph showing average daily activity of same four groups of rats on single food choice diets.

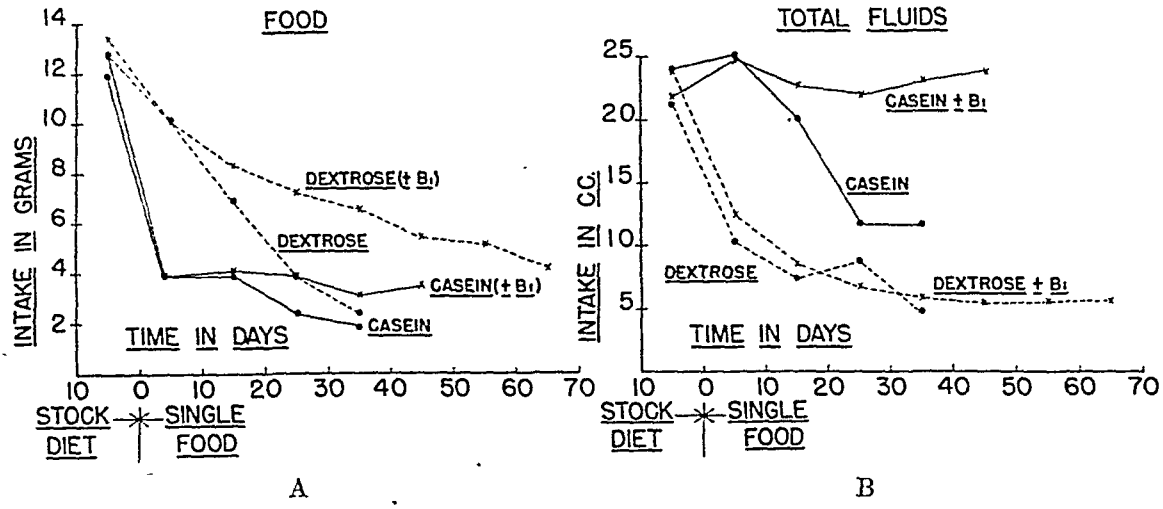

Fig. 2A. Graph showing average daily food intake for rats on single food choice diets.

Group I—dextrose (21 rats). Group II—casein (16 rats). Group III—dextrose + B_1 (12 rats). Group IV—casein + B_1 (14 rats).

Fig. 2B. Graph showing average daily water intake of same four groups of rats.

the previous 10-day period on the stock diet. Later the rats on casein alone decreased their water intake at a rapid and constant rate, while those on casein and thiamine continued to drink just as much fluid as they had on the stock diet.

These records show that thiamine had very different effects on the casein and dextrose rats. The thiamine helped to maintain the water intake of the casein rats at a constantly high level which closely paralleled their intake on the stock diet, while it had no effect whatsoever on the intake of the dextrose rats.

Thiamine intake. Throughout the entire survival period, and most particularly during the last two 10-day periods, the thiamine intake of the rats on casein far surpassed that of the rats on dextrose. Figure 3A summarizes the results. During the first 30 days the rats on dextrose daily drank only about 1.5 cc. of the thiamine solution while the rats on casein drank 8.5 cc. During the following two 10-day periods the rats on dextrose increased their thiamine intake only very slightly while those on casein increased theirs by almost 50 per cent. Progressively as the rats on casein increased their intake of the thiamine solution they decreased their intake of water. Figure 3B, which shows the thiamine intake

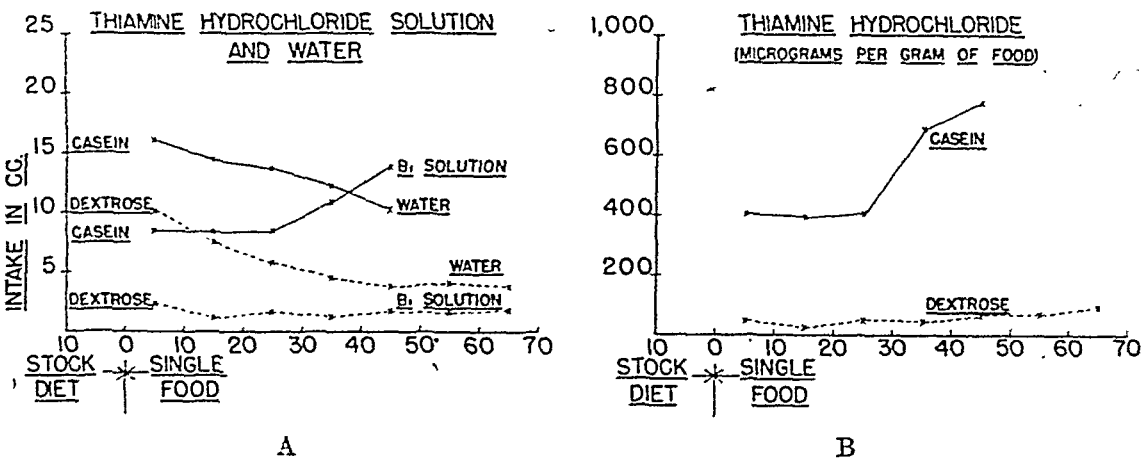

Fig. 3A. Graph showing average daily intake of water and of B_1 solution of rats of groups III and IV.

Fig. 3B. Graph showing average daily intake of thiamine hydrochloride for rats of groups III and IV.

in micrograms per gram of food, brings out very clearly the great difference in thiamine appetite of the rats on dextrose and casein. During the 40 to 50-day interval on the single food diets the rats on casein ingested almost 13 times as much thiamine as did the rats on dextrose. The rats of both groups increased their intake after the middle of the survival period.

Vaginal smears. None of the rats in all four groups showed more than one or two 4 to 5-day cycles of cornified smears after the start of the single food diet, while some went into a state of diestrus immediately. Later the vaginal smears of the rats of groups I and II showed only leukocyte and epithelial cells until the end with the exception of a few which showed constant cornification of the cells after the 30th day. The vaginal smears of the rats on dextrose and thiamine began to show constant cornified cells after having been on the single food diet an average time of 56 days (range 37–72 days), while the smears of the rats on casein and thiamine showed a constant cornification of the cells after an average

time of 35 (15–50) days on the single food diet. Thus the rats on casein and thiamine seemed to develop symptoms of vitamin A deficiency sooner than the rats on dextrose and thiamine. None of the animals developed a keratitis or any of the other symptoms of vitamin A deficiency.

Deficiency symptoms. Apart from the cornification of the vaginal smears none of the rats showed any signs of nutritional deficiency beyond marked emaciation. When they died, even the rats which lived over 70 days had fine coats of hair, clean smooth tails, healthy normal appearing teeth. The rats on casein seemed to have slightly better coats of hair than those on dextrose.

Discussion. In these experiments, in which a protein (casein) or a carbohydrate (dextrose) constituted the sole source of nourishment, the rats ate about one-third as much of the protein as of the carbohydrate. On the basis of results of our previous self-selection studies it may be assumed that when kept under these conditions of qualitative starvation the rats ate just as much of each substance as they were able to utilize. From various single food choice experiments it is known that rats have very little or no appetite for proteins such as gelatin or lactalbumin, which show a deficiency of some of the essential amino acids (4). Rose has shown that addition of the lacking amino acids to a deficient diet increases the daily food intake (5). It would thus seem likely that had we offered the rats a more complete protein, or casein supplemented with tryptophane and cystine, the protein appetite might have been increased.

Of special interest is the fact that despite the much lower food intake the rats lived almost as long on casein as on dextrose. One obvious explanation may be that the rats on dextrose used up most of their energy in running activity. Further, it is possible that with the addition of tryptophane and cystine the casein rats would have lived at least as long as on dextrose. Among other possible limiting factors we may consider difficulties of absorption and the accumulation of nitrogen waste products. We have found that on casein 4 rats which were given access to a 0.2 per cent solution of hydrochloric acid and a 0.5 per cent solution of pepsin lived an average time of 43 days which would seem to be a significant increase in survival time over the 33 day average.

It is possible that the 35 to 45 day survival time may represent the maximum length of time that rats can live without any vitamins, particularly without thiamine. Experiments with supplementary choices of a variety of vitamins, minerals, and enzymes should give an answer to these questions.

The results of the present experiments have demonstrated beyond doubt the importance of the rôle played by thiamine in the utilization not only of the carbohydrate dextrose, but of the protein casein. That thiamine should have so markedly increased the utilization of a carbohydrate agrees with the results of biochemical studies; that it should have such a marked effect also on utilization of a protein is a less expected result. It is known that about half of the casein is ultimately absorbed and metabolized as dextrose. The thiamine might act on this part of the casein. However in the present experiment the casein intake was so small compared to that of the dextrose that the effect of thiamine on the dextrose component of casein could hardly account for the 66.7 per cent increase

in survival time. It is also possible that the larger amounts of thiamine consumed by the casein-fed, as compared with the dextrose-fed, rats represents a need for an acid solution rather than for the vitamin as such.

That thiamine has an effect on the protein appetite was demonstrated in dietary self-selection experiments in which rats had access to a variety of foodstuffs, minerals, and vitamins (6). When they were deprived of all components of the vitamin B complex the rats ate fat, very little carbohydrate, and no protein. When given access to a solution of thiamine they reversed their fat and carbohydrate appetites and ate a small amount of protein (casein). The protein appetite however did not return to its normal level until solutions of riboflavin, nicotinic acid, pantothenic acid, and pyridoxin had also been made available.

According to the results of the present experiments far larger amounts of thiamine are needed for the utilization of casein than of dextrose, especially toward the end of the partial starvation. During the last 10-day period the intake of thiamine was almost 13 times as high in the casein group as in the dextrose group.

By their water intake the rats indicated that far larger amounts of water are needed for the metabolism of casein than of dextrose. The water intake of casein rats remained essentially the same as on the full diet in spite of a very much lowered food-intake. In the casein rats the higher amount of water may be needed to eliminate the greater amount of nitrogen waste products.

The lack of any signs of dietary deficiencies, except possibly for a vitamin A deficiency, requires special comment. Even at the end of 50 to 60 days on the single food casein diet there was no loss of hair, no dermatitis, the coat was in excellent shape. The only change was the marked loss of weight, whereas on a full diet which lacks only one substance, for instance vitamin A, marked deficiency symptoms often appear in 10–15 days. The failure of any deficiency to develop in the single food diets must have been due at least in part to the lowered caloric intake. In the single food experiments the rats were able to adjust their caloric intake closely to their needs, while in the experiments in which they have to eat a full diet and are not allowed to regulate their intake of any individual substance the rats have to take substances which may actually be harmful to them. This brings further evidence for the view that dietary deficiencies are often due not to a lack of calories in the diet, but to an excess.

SUMMARY

1. Sixteen rats which had access to only one foodstuff, casein and water, survived on the average 33 days while 21 rats on dextrose and water survived 37 days. The rats on casein and dextrose lost weight at almost exactly the same rate.

2. The rats on casein immediately became very inactive while the rats on dextrose became even more active than on a stock diet, at least for the first 20 days.

3. The casein rats ate only approximately one-third as much food as the rats on dextrose. Their food intake tended to remain the same from the first to the last days on the single food diet while the food intake of the dextrose rats started

at a higher level, approximately as high as that of the stock diet and then gradually decreased to a much lower level.

4. The 4 to 5 day estrous cycles disappeared almost at once after the start of the experimental diets and did not reappear again.

5. When given access to thiamine hydrochloride 14 rats on casein survived 55 days, that is 22 days or 66.7 per cent longer than on casein alone, while 12 rats on dextrose and thiamine hydrochloride lived 73 days, or 97.3 per cent longer than on dextrose alone. The results of these experiments thus showed under the simplest conditions that thiamine plays an important part in the utilization of protein as well as of carbohydrate.

6. The thiamine had no effect on activity of the casein rats. The rats became less active at once. The rats on dextrose became more active and remained active as long as 60 to 70 days on the diet.

7. The only dietary deficiency which the rats showed was the appearance of constant cornification of the vaginal smears after 50 to 60 days on the diet. Otherwise, the rats appeared to be in excellent shape, except, of course, for the emaciation.

8. By their greatly increased water intake the rats on casein indicated that much more water is needed for the utilization of protein than of carbohydrate.

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THE BLOOD PICTURE OF IRON AND COPPER DEFICIENCY ANEMIAS IN THE RAT

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Received for publication February 4, 1944

The production of an anemia in animals by feeding an exclusive diet of cow's milk has been a valuable technique in extending knowledge of the physiology of iron and copper in the animal body. Schultze (1940) in his review of metallic elements and blood formation has focused attention on one neglected phase of such studies when he stated, "The scarcity of detailed hematological studies on an anemia which can be so well controlled is rather surprising".

Foster (1931) published results of studies on a rather small number of milk anemic rats. His data showed that such rats had erythrocytes smaller than average (microcytosis) and that the hemoglobin content of these cells was low (hypochromia). His statement that "the red cell counts were not much lower in the anemic than in the normal rat" indicates he was dealing with a relatively mild form of milk anemia. Fitz-Hugh et al. (1933) studied the blood and bone marrow pictures of milk anemic rats and showed that the anemia was of the hypochromic type. A microcytosis was found in one rat. Schultze and Elvehjem (1933) studied the reticulocyte response in milk anemic rats fed iron or copper or both. The feeding of iron alone to anemic rats produced no reticulocyte response; copper produced some increase in reticulocytes but not to the extent that iron and copper together produced. Hamilton et al. (1933) published observations on milk anemia in young pigs. These authors found that milk anemia in pigs is hypochromic and normocytic. Dacie (1940) has shown that iron deficiency in the rat leads to an increase in the fragility of the red cells.

The present study reports in more detail the effects of milk anemia on the blood picture of the rat and, in addition, the effects brought about by the feeding of iron or copper to milk anemic rats.

METHODS. Young rats of our colony were made anemic by feeding them an exclusive diet of milk according to the technique of Elvehjem and Kemmerer (1931). The milk was secured from the university herd and milked directly into pyrex glass jars. Iron and copper supplements, when fed, were mixed with a small amount of milk in pyrex custard cups in the morning, and after this was consumed, milk was fed ad lib the remainder of the day. Blood samples from the rats were taken by clipping the tail at bi-weekly intervals until the hemoglobin dropped to about 3 to 5 grams per 100 ml. of blood at which time 1 ml. of blood was taken by heart puncture, mixed with heparin to prevent coagulation and subjected to the following determinations: hemoglobin, red cell count, red cell volume, and reticulocyte count. In addition, blood smears were made for microscopic study. Hemoglobin was determined as oxyhemoglobin accord-

ing to the technique of Sanford and Sheard (1930) with the exception that the transmittance of the diluted blood was determined with a Beckman quartz spectrophotometer at a wavelength of 540 mu. A standard curve of transmittance vs. hemoglobin concentration was prepared using several blood samples in which the hemoglobin was determined by iron analysis (Saywell and Cunningham, 1937). Erythrocyte counts were made in the usual manner using a Spencer bright-line hemocytometer and Trenner automatic pipettes. The red cell volume (centrifuge hematocrit) was determined in a modified centrifuge tube described by Smith (1944). The number of reticulocytes per 1000 erythrocytes was counted in a wet blood smear stained with 1 per cent of brilliant cresyl blue in physiological saline solution. All determinations were run in duplicate. The pipettes calibrated to contain 0.1 ml. and the hematocrit tubes were carefully recalibrated with mercury. The blood smears were stained with Wright's solution and studied under an oil immersion objective.

Rats, hereafter referred to as milk anemic rats, were those animals which subsisted on a diet of cow's milk only. The rats which are referred to as iron-fed are those which developed a severe anemia on an exclusive diet of milk and were then supplemented with 0.5 mgm. of pure iron per rat per day. This iron was derived from iron carbonyl and was found to be spectroscopically free of copper. As is shown later, this iron proved free of copper when tested on anemic rats. The iron was dissolved in a minimum amount of redistilled hydrochloric acid, oxidized to the ferric state with redistilled nitric acid, and made to volume with redistilled water. A good supply of carbonyl iron was given to us by Dr. Oskar Baudisch, but in the meantime we have located a commercial source of carbonyl iron (General Aniline Works, Grasselli, New Jersey) which has also proven to be free of copper. In the majority of cases, there was no significant hemoglobin response in the anemic rats following iron supplementation, in which case a blood sample was taken after four to six weeks for the various determinations. In two rats (nos. 7 and 8 in table 1), there was a significant increase in hemoglobin following the feeding of iron, but the initial rise was followed by a gradual decrease, and a blood sample was taken for study after sixteen and twelve weeks of Fe supplementation, respectively.

The rats designated as copper-fed were those which developed a severe anemia on the milk diet and were then fed 0.05 mgm. of copper per rat per day. The source of copper was electrolytic copper which was dissolved in a minimal amount of redistilled 1:1 nitric acid and made to volume with glass redistilled water. There was no significant rise in hemoglobin in the anemic rats fed copper, and blood samples were taken two to four weeks later for study. It will be recognized that milk anemic rats are Fe and Cu deficient; Fe-fed rats are Cu deficient, and Cu-fed rats are Fe deficient. The rats referred to as normal were litter mates of the anemic rats which received our stock diet.

Results. The hemoglobin response of milk anemic rats when fed iron or copper is given in table 1. The general lack of a hemoglobin response in the Fe-fed anemic rats is interpreted as meaning that the Fe used was free of Cu. The significant hemoglobin response obtained in two of the rats probably means

that these rats possessed a small store of copper which in the presence of iron permitted some regeneration of hemoglobin and that after this source of copper was depleted the hemoglobin decreased in spite of continued iron supplementation. Other evidence that the iron used here is free of copper comes from some unpublished data in which rats weaned from stock dams were fed milk supplemented with 0.5 mgm. of the Fe per rat per day. These rats were unable to

TABLE 1

The hemoglobin response of milk anemic rats when fed iron or copper

IRON (0.5 MGM./DAY)					COPPER (0.05 MGM./DAY)			
Rat	Hemoglobin (gms./100 ml. blood)				Rat	Hemoglobin (gms./100 ml. blood)		
	Initial	2 wks.	4 wks.	6 wks.		Initial	2 wks.	4 wks.
1	3.4	4.4	4.9	3.2	13	4.2	3.0	2.9
2	3.0	2.2	2.3		14	3.8	3.0	3.0
3	3.0	3.4	3.1		15	5.3	4.4	3.3
4	3.4	3.3	2.1		16	3.9	2.7	2.5
5	3.7	3.5	2.6		17	2.9	4.4	4.4
6	4.0	4.1	3.8		18	2.7	5.0	4.6
7	2.9	5.2	5.8	6.7*	19	2.7	2.1	2.1
8	2.9	4.5	7.9	7.0†	20	3.5	6.0	5.1
9	3.1	3.5	3.6		21	3.3	3.2	
10	2.8	4.0	4.1	3.0	22	3.3	2.9	
11	2.8	4.1	3.8					
12	2.4	2.7	3.3	3.8				

* Hemoglobin rose to 11.4 grams and then decreased to 6.7 grams in five weeks.
† Hemoglobin rose to 10.7 grams and then decreased to 5.7 grams in two weeks.

TABLE 2

Summary of the blood pictures of normal and anemic rats

TREATMENT	NO. OF CASES	HEMOGLOBIN	R.B.C.	HEMATOCRIT	M.C.V.	M.C. Hb CONC.	M.C.Hb	RETICULOCYTES
		gms./100 ml.	*millions per cmm.*	%	μ^3	%	$\gamma\gamma$	%
Normal	12	14.91±0.198*	7.421±0.1434	44.71±0.869	60.5±0.35	33.3±0.32	20.2±0.31	3.4±0.56
Milk anemia	21	3.36±0.223	3.175±0.2260	11.55±0.777	36.7±0.70	27.9±1.48	10.7±0.29	9.2±1.56
Fe-fed	12	3.63±0.442	2.776±0.3794	13.51±1.517	51.1±2.89	26.8±0.66	13.7±0.83	7.8±1.12
Cu-fed	10	3.37±0.968	4.255±0.3766	14.58±1.186	34.6±0.97	23.0±0.53	8.0±0.27	23.5±2.96

* Average values with their standard errors.

maintain a normal concentration of hemoglobin which gradually fell to levels of severe anemia.

A summary of the blood picture of normal and anemic rats is given in table 2. This includes the mean hemoglobin values, red blood cell counts (R. B. C.), the centrifuge hematocrits, and the indices calculated from the foregoing values—mean red cell volume (M. C. V.), mean red cell hemoglobin concentration (M. C. Hb. Conc.) and mean red cell hemoglobin content (M. C. Hb.). Also included are the mean reticulocyte counts. The significance of mean differences

was determined by Student's t test. It is noted that all values of the normal rats are significantly different from those of all three groups of anemic rats. The mean cell volume, mean cell hemoglobin concentration, and mean cell hemoglobin of the milk anemic rats are significantly smaller than normal. This shows that in the rat, milk anemia is of the hypochromic and microcytic type which is in agreement with the data of Foster (1931) and the more limited data of Fitz-Hugh et al. (1933). When pure Fe is fed to milk anemic rats, the anemia remains unchanged except that the mean cell volume increases significantly but does not become normal. The anemia still is hypochromic and microcytic but not so severely microcytic. This increase in mean cell volume is easily seen in blood smears under the microscope.

When Cu is fed to milk anemic rats, the type of anemia does not change except to become more pronounced. It should be noted that when Cu is fed to milk anemic rats, there is a significant increase (odds 19:1) in the number of red cells although the hemoglobin level does not change significantly. This is shown more clearly in the mean cell hemoglobin values which are the ratio of Hb/R.B.C. The M. C. Hb is significantly less (odds 99:1) than in the case of milk anemics. This must be due to an uncorrelated rise in the R. B. C. since the Hb has not changed significantly. That Cu stimulates erythropoiesis in milk anemic rats is also supported by the significant rise in the reticulocyte count. Though Cu fed to milk anemic rats stimulates the production of red cells, such cells remain poor in hemoglobin and smaller than normal in size. The stimulating effect of Cu on erythropoiesis in rats has previously been observed by Stein and Lewis (1933).

The percentage of reticulocytes is significantly increased over normal in the case of all three groups of anemic rats, although the increase is relatively small in the case of milk anemics and the milk anemics fed Fe. This is in agreement with the data of Schultze and Elvehjem (1933).

A microscopic study of the blood smears of milk anemic rats showed a pronounced microcytosis and hypochromasia. Basophiles and poikilocytes, while present, were not numerous. In the blood smears of Fe-fed rats, microcytosis was not as pronounced as in the case of milk anemics. The hypochromasia was very evident. No change was noted in the numbers of basophiles or poikilocytes. In the smears of Cu-fed rats, both microcytosis and hypochromasia were pronounced. There was an increase in the numbers of basophiles as compared to the milk anemics and the Fe-fed anemics. Todd and Sanford (1928) state that a reticulocyte appears as a basophile in blood smears stained with Wright's solution.

Grüneberg (1942) has described a new red cell particularly abundant in the blood of anemic flexed-tailed mice which he has termed a siderocyte. This siderocyte is an erythrocyte containing iron which is easily stained with potassium ferrocyanide. We have searched for such cells particularly in the Fe-fed anemic rats but with negative results.

Discussion. The results presented here as well as those previously published by Foster and Fitz-Hugh et al. show that in the rat, a deficiency of Fe or Cu

or both produces a microcytic and hypochromic anemia. In man, it is generally agreed that a simple iron deficiency leads to an anemia of the same type. The authors are not aware of any published data on the blood picture in man where copper alone is the limiting element. As previously mentioned, Hamilton et al. have stated that milk anemia in pigs is hypochromic and normocytic, a picture differing from that of man or rat. Work is now in progress in this laboratory with the rabbit.

As far as hemoglobin synthesis is concerned, it has been generally hypothesized that copper acts merely to catalyze the introduction of iron into hemoglobin, but here as well as in the paper of Stein and Lewis, another rôle of copper is indicated, namely, the stimulation of erythropoiesis and/or stimulation of the release of erythrocytes from the bone marrow.

Some microscopic studies have been made of the bone marrows of iron and copper deficiency anemic rats, but the results are felt to be too preliminary to be presented here.

SUMMARY

A deficiency of iron or copper or both produces an anemia in the rat that morphologically is classified as microcytic and hypochromic. The feeding of pure iron to milk anemic rats leads to a significant increase in the mean cell volume which, however, still remains less than normal. The feeding of copper to milk anemic rats produced a rise in the erythrocyte count which was not accompanied by an increase in hemoglobin.

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ENVIRONMENTAL TEMPERATURE AND VITAMIN K DEFICIENCY

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Received for publication February 14, 1944

Experimental production of fatal hemorrhagic vitamin K[1] deficiency in otherwise normal mammals has not previously been reported, although severe bleeding tendencies have followed ligation of the common bile duct or the production of a biliary fistula (1). Moderate reduction in blood prothrombin has resulted from dietary lack or faulty absorption of the vitamin, but such deficiency has not been severe enough to cause spontaneous hemorrhage and death. This ability to survive on K-free[2] diets has been attributed to synthesis of the vitamin by intestinal bacteria (2), but only moderate prothrombin deficiency has followed the administration of sulfaguanidine to inhibit bacterial growth in the intestines of young rats fed on K-free diets (3).

During the past few years of nutritional study on rats, we have at no time added vitamin K to the diet mixtures. Hemorrhagic states rarely developed in rats of any age so long as they were kept at moderately cool temperatures. In tropical moist heat, however, there was a troublesome incidence of fatal internal hemorrhages,—but only among older rats. Studies in which weanling rats on synthetic diets were observed for 4 to 7 weeks were never complicated by this trouble, nor did hemorrhages occur in older hot room rats fed on Purina chow.

Our attention was forcibly drawn to this hot room hemorrhagic tendency when a large series of 300-gram rats were suddenly shifted from Purina chow to a K-free synthetic diet and at the same time placed in tropical moist heat. This was done late in July, when the rats had already experienced several weeks of Cincinnati heat in the un-conditioned animal quarters. During the third week after the dietary change, 13 out of 32 rats in the hot room died of internal hemorrhage. Addition of vitamin K(2-methyl-1,4-napthoquinone, 5 mgm./kgm.) to the synthetic diet promptly stopped the hemorrhagic tendency and prevented its further appearance in new replacements to the hot room group. Among the rats of an analogous group placed at 68°F., only 2 hemorrhagic deaths occurred before fortification of the diet with the vitamin.

During the cooler weather of October, another series of 300-gram rats were transferred from the un-conditioned quarters to the hot and cold rooms. They were left on Purina chow for three weeks and were then shifted to synthetic diets containing 0, 1, and 2 mgm. of vitamin K per kilo of food. Hemorrhages occurred early in the third week only in the groups getting no vitamin K (3 out of 15 in the hot room and 1 out of 15 in the cold). By the beginning of the fourth

[1] The vitamin K used in these studies was very kindly supplied by the Research Laboratories of S.M.A. Corporation and the haliver oil by the Abbott Laboratories.

[2] Vitamin K has been abbreviated to K in speaking of K-free diets, K-deficiency, etc.

week on the diets, blood coagulability was found to be almost normal, even in the rats on K-free diets.

We next added 0.5 per cent sulfaguanidine to the synthetic diet mixtures and repeated the previous series, allowing 3 weeks for adaptation to the heat and cold (on Purina chow) before changing to the synthetic diets. Hemorrhages

TABLE 1

Blood clotting time with rats on graded K-deficiency

(Diets* contained 0.5 per cent sulfaguanidine)

	RATS KEPT AT 68°F.				AT 90–91 AND 60–70%RH			
VITAMIN K IN FOOD (MGM./KGM.)	0.0	0.3	0.6	1.2	0.0	0.3	0.6	1.2
	sec.	*sec.*	*sec.*	*sec.*	*min.*	*sec.*	*sec.*	*sec.*
After 9 days on diets	175.00 ±23.85		129.00 ±10.79	97.00 ±4.95	5.50 ±1.25		97.00 ±3.94	73.75 ±1.62
After 16 days on diets	245.42 ±59.90	79.00 ±3.05	66.88 ±2.90	70.00 ±2.08	28.65 ±7.12	172.14 ±13.78	85.91 ±3.10	73.33 ±3.66
After 23 days on diets	138.57 ±13.92	77.00 ±2.20	68.50 ±2.46		8.00 ±2.59	99.55 ±4.58	76.91 ±1.78	76.67 ±5.35
After 30 days on diets	65.00 ±1.21	71.67 ±1.95	67.50 ±1.71		2.04 ±0.15	91.67 ±3.64	72.78 ±2.28	78.33 ±4.49
Hemorrhagic deaths	12½%	0	0	0	50%	0	0	0

* Synthetic diets contained:

Sucrose	76 gm./100 gm.
Casein (vit. free)	18 gm./100 gm.
Corn oil	2 gm./100 gm.
Salt mixture	4 gm./100 gm.
Sulfaguanidine	0.5 gm./100 gm.
Haliver oil	1.2 cc./1000 gm.
Thiamine chloride	
cold room	1.5 mgm./1000 gm.
hot room	2.5 mgm./1000 gm.
Riboflavin	4 mgm./1000 gm.
Pyridoxine	4 mgm./1000 gm.
Calcium pantothenate	6 mgm./1000 gm.
Nicotinic acid	25 mgm./1000 gm.
Inositol	1 gm./1000 gm.
p-aminobenzoic acid	0.3 gm./1000 gm.
Choline chloride	
cold room	0.75 gm./1000 gm.
hot room	5 gm./1000 gm.
a-tocopherol	50 mgm./1000 gm.

occurred only in the groups of this series on K-free diets. Thirteen out of 26 hot room rats were so affected (50 per cent) but only 3 out of 24 in the cold (12½ per cent). The hemorrhagic crisis came early in the third week, 1 rat dying of hemorrhage on the 13th day, 7 on the 15th, 5 on the 16th, 2 on the 17th and 1 on the 24th day.

Table 1 shows the mean clotting time values of fresh whole blood obtained

from rats of the various groups of this sulfaguanidine series by cardiac puncture under light ether anesthesia. After nine days on the diets, the rats receiving no vitamin K showed a marked prolongation of the clotting time (greater in the heat than in the cold), while those on 0.6 mgm./kgm. exhibited only a moderate retardation of clotting. After 16 days on the diets, all hot room rats getting no vitamin K showed a marked delay in clotting, 20 per cent of them producing only a very weak clot in 24 hours; about a third of the analogous cold room group exhibited a normal clotting time. With 0.3 mgm. of the vitamin per kilogram of diet, the cold room rats manifested only a very slight clotting delay, much less than was shown by the analogous hot room group. Clotting times were normal on the 16th day with 0.6 mgm./kgm. in the cold and 1.2 mgm./kgm. in the heat.

Clotting tests made after 23 days on the diets showed a marked delay still in effect among the hot room rats on K-free diets, but only a moderate delay among the analogous cold room rats or those on 0.3 mgm./kgm. in the heat. Normal clotting times were shown by those on 0.3 mgm./kgm. in the cold and 0.6 mgm./kgm. in the heat. After 30 days on the diets, all cold room rats showed normal clotting times while there was still moderate delay in the hot room rats on K-free diets and a slight delay with 0.3 mgm./kgm.

Hemorrhages in the K-deficient rats seemed especially prone to occur in 2 general regions: *a*, in the seminal vesicles, around the bladder, or along the venous plexus draining the testicle, and *b*, around the superior mesenteric vein in the upper abdomen. There were 16 hemorrhages in the former general region and 5 in the latter. One rat had a hemorrhage extending from the lower jaw down into the thorax behind the right pleura, and another showed subcutaneous hemorrhage in the right rear foot as well as external bleeding from one toe. No explanation is offered for this limited distribution of the fatal hemorrhages, nor were any similar studies made on female animals.

Discussion. It seems clearly evident that complete K deficiency in adult rats assumes a much greater severity in tropical heat than in temperate coolness. The hemorrhagic death rate is roughly 4 times higher in the heat and the retardation of clotting uniformly greater. Twice as great a dietary content of the vitamin is needed to secure normal clotting in the heat. This does not mean that the absolute K requirement is twice as high in the heat, for these rats eat only about 70 per cent as much food per day as is consumed by those in the cold. It does indicate, however, that the K requirement *per gram of food* is twice as high in the heat as in the cold.

Our findings suggest a convenient method of producing severe K deficiency in mammals, with a high death rate from internal hemorrhage. This had been accomplished previously only by surgical interference with biliary drainage into the intestine. As little as 0.3 mgm. of the vitamin per kilogram of food protects rats against hemorrhage and death in either heat or cold, even when the diet includes 0.5 per cent sulfaguanidine. Complete prevention of prothrombin deficiency seems to require about 1 mgm./kgm.

We have no explanation to offer for the rapid disappearance of the prothrombin deficiency while the rats are still maintained on the K-deficient diet containing

0.5 per cent sulfaguanidine. Although it may well be that the K-producing bacteria in the intestine become adapted to the sulfaguanidine and return to normal activity, our studies were not expanded to cover this point.

Blood taken from the heart of rats prostrated by internal hemorrhage was found to contain even more than the normal amount of fibrinogen and to exert no inhibitory influence on the clotting of normal blood. Our findings thus confirm the conclusions of others (4) that the only abnormality is a prothrombin deficiency.

No explanation can yet be offered for the greater severity of K deficiency in animals adapted to tropical heat, although it is possible that the heat in some way disturbs the production of the vitamin by the intestinal bacteria. Statistics on the most clear-cut human K deficiency (hemorrhagic disease of the newborn) indicate a much higher prevalence in the states bordering the Gulf of Mexico than in those of the North. In a recent limited survey of hospital statistics, we found an incidence of 0.6 per 1000 among some 25,000 infants delivered in Cincinnati hospitals and 2.8 per 1000 among 78,000 live-births in hospitals of New Orleans, Houston and Galveston. Perhaps when proper search is made, K deficiency will be found rather widely prevalent among tropical residents.

SUMMARY AND CONCLUSIONS

Severe and highly fatal vitamin K deficiency can be produced in rats adapted to tropical heat by using K-free synthetic diets containing 0.5 per cent sulfaguanidine.

This deficiency is most severe and fatal early in the third week of such feeding and disappears almost entirely by the fifth week.

Animals adapted to tropical heat seem definitely more prone to severe manifestations of K deficiency than do those kept in temperate coolness, and the K requirement *per gram of food* is twice as high for rats in the heat as for those in the cold.

A preliminary survey of hospital statistics on bleeding in the new-born shows this type of human K deficiency to be 4 times more prevalent among infants born in the Gulf States than among the northern-born.

Fatal hemorrhages in male rats on K-free diets are especially prone to occur in the genital system and around the superior mesenteric vein.

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A CHART FOR THE ESTIMATION OF THE RED CELL MASS OF DOGS FROM THE JUGULAR HEMATOCRIT VALUE AND THE BODY WEIGHT

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Received for publication February 17, 1944

An estimation of the mass of red blood cells in the circulation is frequently necessary in experimental work. Studies related to the excretion of bilirubin, urobilinogen and other materials derived from red cell metabolism are often meaningless unless the actual amount of source material giving rise to these end products can be evaluated. It is also sometimes desirable to be able to determine the total circulating amount of certain constituents of the red cell. In such instances it is necessary to know not only the concentration of the constituents but also the mass of cells in the blood stream. The latter determination by direct methods is ordinarily inexpedient since it involves one of several somewhat involved techniques. The estimation of the cell mass indirectly by measurement of the plasma volume with one of the dye dilution methods followed by application of plasmatocrit values for conversion to blood volume, followed by subtraction of the determined plasma volume as a means of arriving at a value for red cell mass is not only almost as time consuming as the direct methods but it yields incorrectly high values (1).

Elsewhere we have reported values for red cell mass which were obtained by a modification of the Welcker viviperfusion method which affords a direct and accurate method for determining the true cell mass but involves sacrifice of the animal. It was found that when these values obtained from the perfusion of fifteen animals varying in weight from four to eighteen kilograms were plotted against body weight a fairly representative straight line could be drawn through them by the method of least squares. These values were corrected to an arbitrary normal value corresponding to a red cell hematocrit reading of 45 per cent by the assumption that the hematocrit value was a linear function of the red cell mass (2). It was then possible to replot the cell volumes as a function of the jugular hematocrit making this same assumption. The results are shown in figure 1 below which is constructed to show the red cell mass corresponding to various weights of dogs at increments of 1 kilogram and at hematocrit levels ranging from 10 per cent to 55 per cent.

The validity of this chart was tested by comparing the red cell volumes obtained by its use with those arrived at directly for the same animals using the isotope-tagged donor cell method reported earlier (1). In general the agreement was within ten per-cent or better and it would seem that the use of such a simple chart might be justified for many types of work in which a high degree of accuracy is not required. In any case the results obtained by its use would be much closer to the true values than those obtained indirectly by the dye tech-

nique for plasma volume which involves the false assumption that the jugular hematocrit value is representative of the average body hematocrit relationship.

The dogs on which the curves giving rise to this chart were based were all normal or chronically anemic animals, in the latter instances there being sufficient time allowed to elapse before perfusion for equilibration of the cell-plasma fractions to have taken place. Obviously such a chart cannot be used to determine the red cell mass under conditions where the circulation is not adjusted to the needs of the animal such as might obtain following acute hemorrhage or under conditions of shock, inanition, dehydration, cardiac failure, etc.

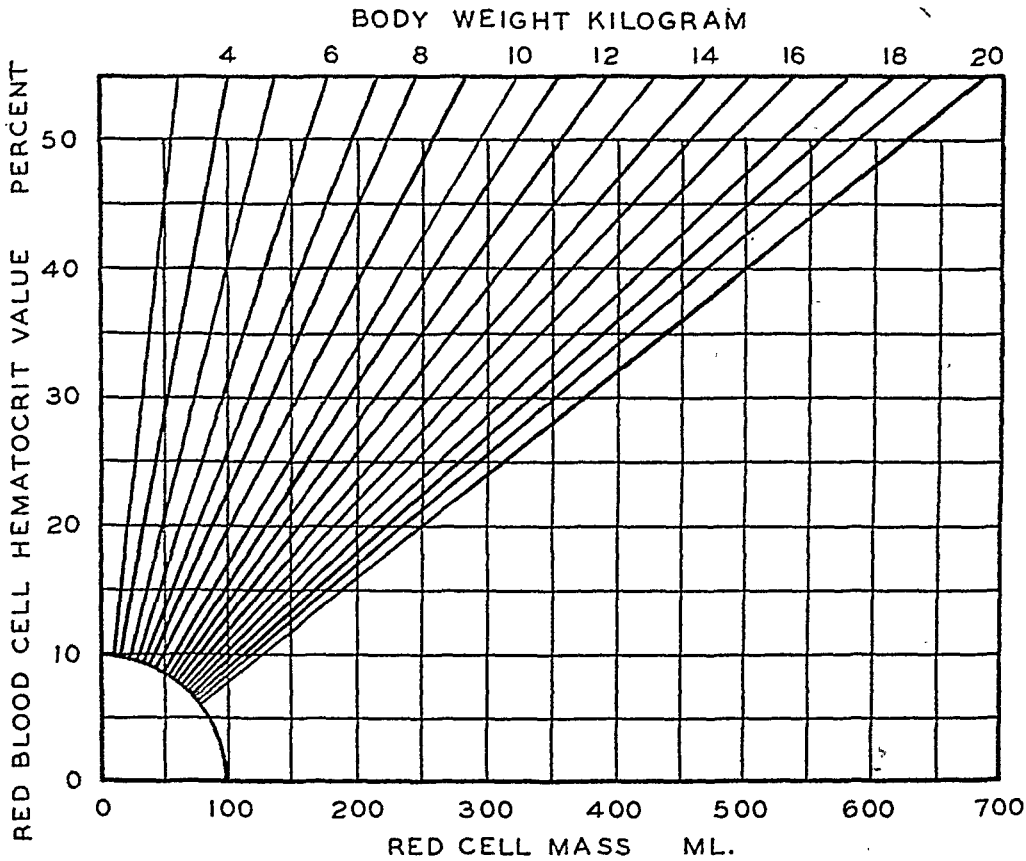

Fig. 1. Follow the constant weight line corresponding to the dog's body weight in kilograms toward the origin until it intersects a horizontal line corresponding to the hematocrit reading and then read directly below on the abscissa the red cell mass in milliliters. For example, a 15 kgm. dog with a hematocrit value of 33 per cent would have a red cell mass of 300 ml.

SUMMARY

A chart is presented which allows the rapid estimation of the red cell mass of dogs, given the body weight and the red cell venous hematocrit value. At an arbitrary normal hematocrit value of 45 per cent the dog has about 27 ml. of red cells per kilogram of body weight.

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EFFECT OF PURIFIED RATIONS ON DECIDUOMAL FORMATION IN THE RAT

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Received for publication February 17, 1944

The purpose of the present communication is to report the effect of purified rations on the morphology of deciduomata in the pseudopregnant rat. Deciduomata were produced in pseudopregnant rats raised to maturity on a purified ration adequate for growth and reproduction and compared morphologically to those observed in littermate controls similarly treated but maintained on a natural food ration.

PROCEDURE AND RESULTS. Twelve female rats weighing 30 to 35 grams were weaned at 21 days of age and placed on the following purified ration[1]: sucrose 73.2, Vitamin Test Casein[2] 22, 1(−) cystine 0.3, and Sure's Salt Mixture no. 1[3] 4.5. To each kilogram of the above were added 20 mgm. thiamine hydrochloride, 20 mgm. riboflavin, 20 mgm. pyridoxine hydrochloride, 100 mgm. calcium pantothenate, 100 mgm. nicotinic acid, 200 mgm. p-aminobenzoic acid, 500 mgm. inositol, 1200 mgm. choline chloride, and 5 mgm. 2-methyl-napthaquinone. Daily supplements of 800 mgm. corn oil (Mazola), 0.5 mgm. alpha-tocopherol, and an A-D concentrate[4] equivalent to 50 U.S.P. units of vitamin A and 5 U.S.P. units of vitamin D were also administered. Animals were kept in metal cages with screen bottoms to prevent access to feces; and sufficient food was administered to assure ad lib feeding. In addition to the above 10 littermate controls were placed at weaning on a natural food ration supplemented once weekly with lettuce.

At four months of age animals showing fully cornified vaginal smears were selected on the first day of an experiment. The cervix uteri of each rat was stimulated for 1 or 2 seconds by an alternating current of 60 volts. Four days after the cervical stimulus was applied the endometrium of the right uterine horn was traumatized by inserting a silk thread for a distance of 1 or 2 cm. through the uterine lumen. Animals were autopsied four days post traumatization, the uteri fixed in Bouin's solution, and sections prepared stained with hematoxylin and eosin[5].

[1] Growth and reproduction of female rats raised to maturity on this ration were not inferior to those observed in littermate controls maintained on a natural food ration. Mothers failed however to suckle their young.

[2] S.M.A. Corporation, Chagrin Falls, Ohio.

[3] Sure, Barnett. J. Nutrition 22: 499, 1941.

[4] Nopco Fish Oil Concentrate, assaying 800,000 U.S.P. units vitamin A and 80,000 U.S.P. units vitamin D per gram.

[5] We wish to express our sincere appreciation to Dr. B. M. Allen of the University of California at Los Angeles for making available material and equipment for histological studies.

All animals exhibited well developed deciduomata at the site of traumatization but a distinct difference was observed between the two groups in the morphology of these structures. In the natural food series a single stimulus along several centimeters of the uterine lumen resulted in the development of several distinct deciduomata corresponding grossly to fetal sites of a similar stage of pregnancy. A similar stimulus to animals on the purified ration resulted however in diffuse deciduomata frequently extending as a single homogeneous swelling from cervix to oviduct. In no instance on the purified ration was the decidual tumor as restricted and circumscribed as that observed in animals on a natural food ration. Histologically no marked differences were observed in cross section in the morphology of deciduomata in either of the dietary groups. The proportion of mesometrial to antimesometrial stroma cells which Rothchild, Meyer and Spielman (1) believe to be indicative of the relative amounts of estrone and progesterone present were uniform throughout. The uterine lumen was invariably found in a central position; its epithelium when surviving was low cuboidal but in fully half the cases the surface epithelium of the lumen was lacking and marked extravasation into the lumen had taken place. In contrast to the localized and circumscribed swellings of the natural food series, deciduomata in the purified series constituted a homogeneous structure retaining its microscopic characteristics relatively unchanged through its entire length. No spontaneous deciduomata were observed in the untraumatized horn of any of the rats regardless of the dietary regimen.

Attempts to prevent diffuseness of decidual response by means of a single dietary supplement have been unsuccessful. Six litter mates of the above series were placed at weaning on a diet identical to the purified ration above. On the day of cervical stimulation wheat germ oil[6] was substituted for the corn oil of the ration. Deciduomata produced on this ration were as diffuse as those observed in animals receiving corn oil throughout. In another series six female rats were placed at weaning on a purified diet similar to the above but with the crystalline vitamins omitted and with brewer's yeast replacing sucrose as ten per cent of the ration. Deciduomata produced in this series under similar conditions to the above were equally diffuse.

Diffuse deciduomata were first described by Evans (2) as occurring in pseudopregnant rats maintained on a vitamin E-deficient diet. Unpublished work by the author (3) indicates however that this condition was not due to deficiency of vitamin E inasmuch as administration of alpha-tocopherol failed to prevent diffuseness of deciduomata formed on a vitamin E-low diet. In the present series wheat germ oil was similarly ineffective. It would appear that some dietary factor(s) other than that employed in the purified rations above, present in a natural food ration but absent in significant quantity in wheat germ oil and yeast, is necessary for restricting the growth of deciduomal tumors in the rat.

SUMMARY

Traumatization of the endometrium of pseudopregnant rats raised to maturity on a purified ration resulted in the formation of diffuse deciduomata in contrast

[6] Viobin Corporation, Monticello, Illinois.

to the circumscribed growths in animals similarly treated but maintained on a natural food ration. Addition of wheat germ oil or brewer's yeast failed to prevent diffuseness of deciduomal response. It is suggested that some dietary factor(s) present in natural food but absent in the purified rations employed is necessary for restricting the growth of deciduomal tumors in the rat.

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KINETICS OF THE DISAPPEARANCE OF GALACTOSE FROM THE PLASMA AFTER A RAPID INTRAVENOUS INJECTION

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Received for publication January 14, 1944

In studies of the intermediary metabolism of galactose the presence of a simultaneous galactosuria has been an obstacle to the interpretation of several experimental results (Deuel, 1936). In order to avoid this obstacle investigators have either used an animal species with kidneys slightly permeable to galactose or resorted to a nephrectomized animal. The results of these procedures, however, cannot be immediately extended either to other animal species or to the intact animal.

There is need, therefore, for a method of calculating 1, the amount of galactose present in the body at any given time during the experiment, and 2, the rate at which galactose is removed from the plasma by processes other than excretion. The first depends on the existence of a volume of distribution, that is, a fixed volume of body fluids which hold galactose in equilibrium with the plasma (Dominguez, 1934). The second requires an analysis of the changes in the concentration of galactose in the plasma. Such an analysis incidentally determines the volume of distribution.

In general, the equation of the rate of change of the concentration in the plasma is quite complex, for it contains both the rate at which the substance enters the plasma and the rate at which the substance leaves the plasma. This complex situation can be simplified by injecting the substance as rapidly as possible into the vein. Under these conditions, both the time taken by the injection and the phenomena occurring in this interval can be neglected. The change in the concentration of the substance in the plasma will then reduce simply to a fall in concentration, and the equation of the rate of fall in concentration will contain only the rates of the processes that produce this fall.

In the particular case of galactose this fall in concentration is brought about by 1, diffusion from the plasma into other body fluids; 2, excretion, and 3, chemical transformation. Diffusion, a purely physical phenomenon, needs no special comment. In regard to excretion, only renal excretion need be considered because galactose is not excreted in significant amounts by channels other than the kidney. As to the chemical transformation, whether oxidation, polymerization, direct conversion into glucose, etc., only the rate of the first step of this transformation is required. This rate, that is, the rate at which galactose is removed from the plasma by or for conversion, will be referred to as the rate of utilization.

It is the purpose of this paper to analyze the fall in the concentration of galactose in the plasma into its different components and, from data on both excretion and plasma concentration, to determine the volume of distribution, the

rate of utilization, and the relation of the rates of excretion and utilization to the plasma concentration.

It is hoped that the results of this study will be of use to investigators of galactose metabolism. Moreover, because of the generality of the reasoning on which the analysis of the plasma curve is based, the method given here is expected to be applicable to other substances and to other processes.

EXPERIMENTAL PROCEDURE. The experiments were carried out in five female mongrel dogs weighing from 11.4 to 22.7 kgm. The routine diet of these dogs consisted of Purina Dog Chow and occasional bones. No food was given to the animals the day before the experiment and during the experiment. In order to ensure adequate diuresis, water was available to them at all times and, in most instances, was given by stomach tube during the experiment.

A weighed amount of *d*-galactose (C. P., Pfanstiehl), varying between 3.56 and 65.46 grams, was dissolved in freshly distilled water and the solution sterilized by filtering through a Seitz filter. The solution was injected into a vein as rapidly as possible. The quantity of fluid injected varied between 19.9 and 217 cc. and the concentration of the solution between 15.4 and 47.8 grams of galactose per 100 cc. of solution. In 20 of the 23 experiments the time consumed by the injection varied between ½ and 5 minutes; in one experiment it took 7 minutes (2 injections); in another 9½ minutes (2 injections); and in still another 19 minutes (2 injections). In most experiments a jugular vein was used for the injection. The only ill effects, observed especially with the larger doses, were tremor of the whole body and flush of the skin and of the mucous membranes. If they occurred at all, both effects appeared during or shortly after the injection but disappeared in twenty minutes or less.

The samples of blood were withdrawn from a jugular vein at varying times after the injection. The times were arranged so that the intervening concentration could be obtained by linear interpolation with an error not larger than the error of the chemical method.

The bladder was catheterized with a no. 14 coude catheter. The urine was collected directly into graduated cylinders and the volume read at room temperature. When the volume of urine was small, the washings of the catheter were also analyzed. The intervals of collection were selected so as to justify the use of the mean rate of excretion in a given interval as the rate of excretion at the middle of the interval.

A number of samples of blood and urine were analyzed before and between the experiments in order to determine the concentration of endogenous reducing substances given by the chemical methods employed.

Chemical methods. The blood was withdrawn in amounts varying from 10 to 20 cc. and was collected in bottles containing approximately 30 mgm. of dried potassium oxalate. The plasma was separated by centrifugation usually within one or two hours after the blood withdrawal. In a few experiments the late blood-samples were kept in the refrigerator (6°C.) overnight, before centrifugation, but repeated checks showed no essential change in the concentration of reducing substances of the plasma. For the determination of the reducing substances of

the urine and the non-fermentable reducing substances of the plasma, the method of Folin and Wu (1920) was used, with *d*-galactose standards of appropriate strength. The yeast-fermentable reducing substances of the plasma were removed by the method of Blanco (1928). The Klett Biocolorimeter was used for the color matching. The determinations were made in duplicate, sometimes in triplicate, on samples renumbered by an independent worker. In some of the later experiments the Klett readings were checked with the Evelyn or the Klett-Summerson photoelectric colorimeter.

TABLE 1

Protocol of experiment 5, dog 1

URINE			PLASMA	
Interval of collection (*9:30 a.m.–3:43 p.m.*)	Volume	Concentration of reducing substances	Time of blood withdrawal	Concentration of non-fermentable reducing substances
	cc.	*mgm./100 cc.*		*mgm./100 cc.*
9:30 – 9:47	219.5	3393	9:51 a.m.	997
9:47 –10:11.5	181.0	4800	10:16	687
10:11.5–10:35.5	114.5	5575	10:39	551
10:35.5–10:57	80.5	5075	11:01	406
10:57 –11:20	53.7	6518	11:23	332
11:20 –11:48.5	41.8	7093	11:51.5	234
11:48.5–12:21	65.0	3755	12:28 p.m.	138
12:21 – 1:13.5	139.0	1411	1:18	63.3
1:13.5– 1:52	23.7	2923	1:56	37.7
1:52 – 2:27	7.0	2285	2:31	14.1
2:27 – 3:11	97.2	116	3:13	13.3
3:11 – 3:43	44.0	75.2	3:46	9.5

Food was withdrawn 24 hours before the experiment. From 9:26.5 to 9:29 a.m., 91.3 cc. of a 36.2 per cent solution of *d*-galactose (Pfanstiehl) was injected into a leg vein, and from 9:35 to 9:36 a.m., 68.2 cc. of the same solution was injected into a jugular vein. Flush and trembling developed in the course of the injections. Trembling had ceased by 9:40, and flush by 9:50 a.m. A small amount of urine was lost accidentally at the first catheterization. At 8:31, 11:25 a.m. and 1:59 p.m. water was given by stomach tube, 400 cc. each time. The animal was fed at 3:20 p.m. when the experiment was practically over. At 8:08 the following morning, the urine voided during the night and washings of the cage floor (290 cc.), together with 35 cc. of catheterized urine, were pooled and analyzed. The concentration of reducing substances in this sample was 209 mgm. per 100 cc. and the rate of excretion in this long interval 0.69 mgm. per minute.

Data. An illustrative protocol is reproduced in table 1. The weights of the dogs on the day of the experiment, the quantities of injected galactose, and the amounts of galactose excreted in the indicated intervals of time are given in table 2. In order to save space, the calculated amount of excreted galactose and the per cent difference between the excreted and calculated amounts are also given in table 2.

The endogenous values of both the non-fermentable reducing substances of the plasma and the reducing substances of the urine are essentially those given

in another paper (Dominguez, Goldblatt and Pomerene, 1937). The mean values for the plasma, expressed in milligrams of galactose per 100 cc., are the following: dog 1, 10.6; dog 2, 13.6; dog 3, 9.2; dog 4, 8.0; and dog 5, 9.6. The

TABLE 2

Quantities injected and amounts excreted

DOG NO.	EXPT. NO.	WEIGHT	QUANTITY INJECTED G	AMOUNTS EXCRETED IN INDICATED TIMES				PER CENT DIFFERENCE $100 \times \frac{E-I}{E}$
				Found		Calculated		
				t	E	t_1	I	
		kgm.	*gm.*	*min.*	*gm.*	*min.*	*gm.*	
1	1	13.9	3.56	98.4	1.034	99.1	1.075	−3.95
	2	13.6	6.82		2.327			
	3	11.3	7.83		2.258			
	4	13.0	10.65	160	4.189	155.5	4.375	−4.45
	5	14.1	57.74	341	38.235	314	40.493	−5.9
	6	13.6	59.71	1505	33.410	∞	33.600	−0.57
	7	22.7	63.79	374	40.144	366.6	40.671	−1.31
2	1	16.6	4.20		0.987			
	2	16.8	10.12	228	3.884	227.4	3.595	+7.45*
	3	16.8	17.62		5.560			
	4	16.3	31.34	284.5	15.884	284.5†	15.193	+4.35
	5	16.8	36.64	356.5	19.858	356.5†	20.087	−1.15
	6	16.6	65.46	421	43.971	431.0	42.960	+2.35
3	1	15.7	13.40	205.5	6.448	182.7	6.448	0
	2	16.3	50.14	304	32.032	284.4	37.515	−19.02*
4	1	11.6	24.98	282	17.464	297.8	18.224	−4.35
	2	11.4	38.42	370	27.656	372.4	29.949	−8.3*
	3	11.3	42.68		30.885			
5	1	15.9	3.96	115	0.839	109.4	0.896	−6.7
	2	12.0	28.68	307	16.218	282.6	17.437	−7.5
	3	17.7	39.14		22.31			
	4	15.4	55.34		38.25			
	5	15.4	55.49		36.67			

* In these experiments there is a large discrepancy between the amount excreted in the first interval of collection and the amount calculated for this interval. If this interval is omitted, the per cent differences become: +0.22 (expt. 2, dog 2), −3.58 (expt. 2, dog 3), and −3.50 (expt. 2, dog 4).

† The times t_1 of experiments 4 and 5, dog 2, are 466 and 472 minutes, respectively. However, the integration cannot be carried to these times because the catheterization of the bladder was stopped at the times given.

mean values for the rate of excretion, in milligrams per minute, are, in the same order: 0.56; 0.58; 0.79; 0.48; and 0.47. In this paper the mean values of the blanks will be treated as constants.

Rate of excretion. If the rates of excretion and the plasma concentrations

are plotted on ordinary co-ordinate paper with the times as abscissae and if the points of each respective set are joined by segments of straight lines, the resulting curves fall regularly from the beginning to the end of the experiment. By graphic linear interpolation the rates of excretion y can be paired with the corresponding plasma concentrations x. If the data (y, x) are now plotted in the ordinary way, the relation between these quantities is, in most instances, approximately linear from the endogenous level up to the highest plasma concentration reached in these experiments. As an example, we have plotted the points obtained from four experiments (fig. 1). The linear relation between the plasma concentration x and the rate of excretion y is evident.

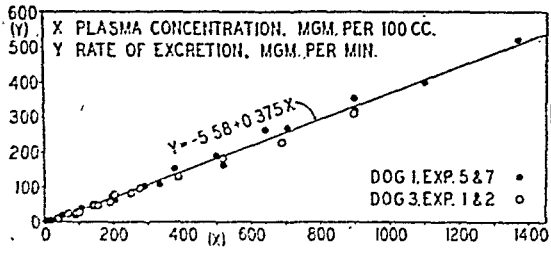

Fig. 1

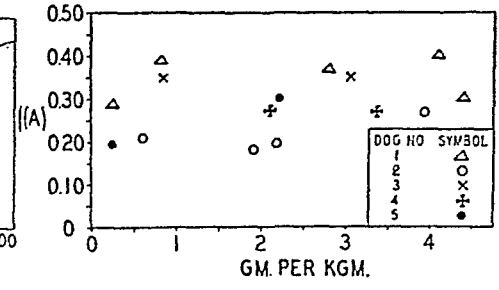

Fig. 2

Fig. 1. Relation of the rate of excretion of reducing substances to the plasma concentration of non-fermentable reducing substances. Forty-two pairs of observations from four experiments on two dogs have been plotted. Below $x = 60$, ten points cannot be seen distinctly because of overlapping. The line was fitted by the method of least squares. The standard error of the slope is 0.00476. This line does not fit well the mean endogenous point of dogs 1 and 3. The line passing through this mean and the centroid of all the other points is $y = -2.95 + 0.366\ x$. However, the difference between the slopes of these two lines is 0.009, and, lying within twice the standard error of the slope of the first line, cannot be considered significantly different.

Fig. 2. Relation of the excretion constant A to the quantity injected per kilogram of body weight. In most of the experiments the excretion constant A (table 3) is independent of the quantity injected. The slight correlation in the experiments on dog 2 and the apparently greater correlation in the experiments on dog 5 are probably due to the effect of diuresis.

Since the endogenous pair is used in the calculation of the slope, we can make this pair the origin of co-ordinates and write the relation between the rate of excretion of galactose η and the plasma concentration of galactose ξ in its simplest form,

$$\eta = A\xi. \tag{1}$$

In this equation, the excretion constant A can be interpreted as the rate of excretion of galactose per unit plasma concentration of galactose.

Strictly speaking, A is constant only when diuresis is constant. However, when water is given in several doses during the experiment, a systematic variation in diuresis is prevented, and very low diureses are not likely to occur. Under these circumstances, a mean value of A is maintained throughout the experiment

and the slope of the line is constant (fig. 1). On the other hand, when water is not given during the experiment (as happened inadvertently in expts. 3, 4 and 5, dog 5), diuresis falls continuously, the value of A diminishes, and the relation between the rate of excretion and the plasma concentration ceases to be linear.

Restricting ourselves to the fifteen experiments in which A is constant, we can see in figure 2 that there is no correlation between the excretion constant and the dose (data of tables 2 and 3).

Equation of the plasma concentration curve. As stated in the introduction, the rate at which galactose disappears from the plasma is equal to the sum of the rates of diffusion, excretion and utilization. After equilibrium between the plasma and the tissues is reached, the rate of disappearance is equal to the sum of only the rates of excretion and utilization. If we call ζ the quantity of galactose present in the body at a given time, η the rate of excretion and u the rate of utilization, we have, after equilibrium,

$$-\frac{d\zeta}{dt} = \eta + u. \tag{2}$$

The quantity ζ is equal to the product of the plasma concentration ξ and the volume of body fluids which hold galactose in equilibrium with the plasma. If we call this volume V, then $\zeta = V\xi$. Since the volume V can be assumed to remain constant during the experiment, equation 2 becomes

$$-V\frac{d\xi}{dt} = \eta + u. \tag{3}$$

Inserting in equation 3 the expression for η from equation 1 we get, finally,

$$-V\frac{d\xi}{dt} = A\xi + u. \tag{4}$$

This equation shows the relation that exists between the curve of the plasma concentration ξ, the slope of this curve $\frac{d\xi}{dt}$, and the rate u at which galactose is removed from the plasma by processes other than excretion.

The rate u can now be determined in two ways: 1, assume a mathematical expression for u and test, after integration of equation 4, how closely the plasma data are satisfied, or 2, assume a mathematical expression for the plasma curve and, after performing the operations indicated in equation 4, compute u at a series of points. The first method was used to give the rate u from the initial value of the plasma concentration down to small values of this concentration—20 mgm. per 100 cc. or less. The second was used to show the trend of the curve u at the low values of the plasma concentration.

The steps in the calculations can be simplified by the following considerations.

In the first place, the plot of log ξ and the time (fig. 3, upper right) shows that, in the beginning, when the difference in concentration between the plasma and the tissues is greatest, there is no initial drop in concentration which is due to

diffusion. In order to determine the early stages of this diffusion it would be necessary to make observations within the first five or ten minutes after the injection. For the purpose of the present study, however, this small interval of time

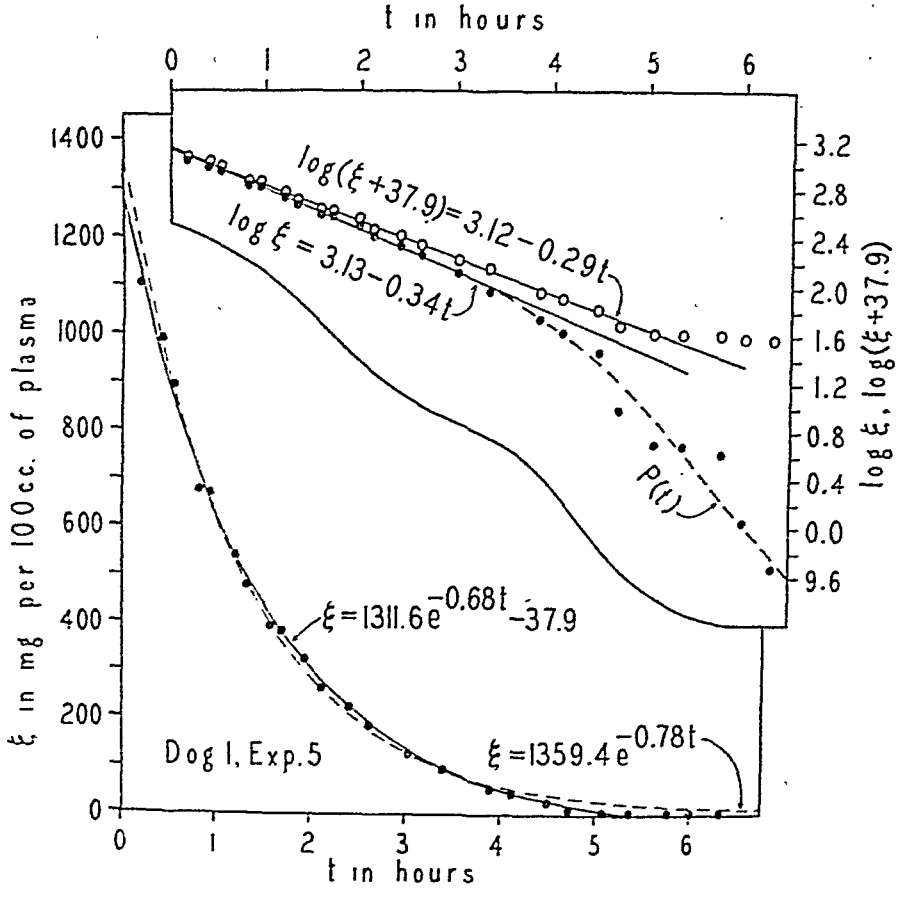

Fig. 3. Time curve of the plasma concentration ξ and rate of excretion η of galactose in experiment 5 on dog 1. The plasma concentrations have been plotted after subtraction of the endogenous blank. The rates of excretion, after subtraction of the blank, have been multiplied by $1/A$ (equation 1) in order to bring them to the plasma level. These two sets of points have been drawn without distinction. The simple exponential curve (broken), which fits the points down to the time $t = 4$, passes systematically above the points beyond $t = 4$. This situation is better demonstrated in the upper right corner of the figure, where the logarithms of the same experimental data have been plotted. The first part of the plot (black circles) follows a straight line down to $t = 3.2$. From $t = 3.2$ to $t = 6.2$ the points follow the broken curve $P(t)$. This curve represents a polynomial of the fourth degree in t, fitted to the mean position of these points. In this experiment, the constant p of equation 6 is equal to 37.9. The straight line of log $(\xi + 37.9)$ and the curve of ξ corresponding to equation 6 (continuous) follow the points down to $t_1 = 5.2$.

can be neglected. Therefore, equation 4 can be assumed to hold from the beginning of the experiment.

In the second place, the same plot of figure 3 shows that the logarithms of ξ do not decrease linearly throughout the experiment. This non-linearity means, of course, that the rate of utilization is not proportional to the plasma concentration, except perhaps in the early part of the experiment. A closer approximation

is obtained by assuming for the rate of utilization a linear function of the plasma concentration, namely,

$$u = a + B\xi. \tag{5}$$

Substituting this expression for u in equation 4 and integrating, we get

$$\log [a + (A + B)\xi] = \log [a + (A + B)\xi_0] - \frac{A + B}{V} \cdot \log e \cdot t,$$

or, more simply,

$$\xi = qe^{-kt} - p, \tag{6}$$

where

$$q = \xi_0 + p, \tag{7}$$

$$k = \frac{A + B}{V}, \tag{8}$$

$$p = \frac{a}{A + B}, \tag{9}$$

$$V = \frac{G}{\xi_0}, \tag{10}$$

and where e is the base of natural logarithms, t the time, and, as before, ξ the plasma concentration and V the volume of distribution. The initial plasma concentration ξ_0 means the concentration at the time $t = 0$. In these experiments, the time zero has been identified with the beginning of the injection. As far as the plasma concentration is concerned, this identification is arbitrary, because during the injection and even for a short time after the injection the concentration of the substance in the plasma varies greatly in different parts of the circulatory system. However, as long as observations are made on excretion, it is better to consider that the experiment begins at the beginning of the injection.

Computations. The constants q, k and p in equation 6 were computed by the method of least squares as modified by Scarborough (1930). The values of the constants are given in table 3.

The degree of approximation obtained was ascertained both by examining the closeness with which the calculated curves follow the data and by comparing the amount actually excreted with the amount calculated. The curves fit the data down to small values of the plasma concentration, below 20 mgm. per 100 cc. An example of the fitting is shown in figure 3. The amounts calculated are given in table 2. Even though the curves were not fitted down to $\xi = 0$, the integration was carried to the time t_1 corresponding to zero plasma concentration ($t_1 = \frac{\log q - \log p}{k \cdot \log e}$) because the error thus committed is negligible. The mean difference between the amount found and the amount computed, expressed in per cent of the former, is -3.46, and the average difference, irrespective of sign,

±5.48. The largest single errors are found in the first fifteen minutes of the experiments (table 2).

The difference between the amount excreted in the time t_1 (table 2) and the amount excreted in 24 hours, expressed in per cent of the former, varies between −5.02 (expt. 1, dog 1) and +2.44 (expt. 2, dog 2), with a mean of −1.21 and an average of ±2.07.

TABLE 3

Numerical values of the constants in the equations of the rate of excretion η, plasma concentration ξ, and rate of utilization u

DOG NO.	EXPT. NO.	$\eta = A\xi$	$\xi = qe^{-kt} - p$				$u = a + B\xi$	
		A	q	k	p	V	a	B
		Excretion constant				Volume of distribution		Utilization constant
		100 × cc. per minute	*mgm. per 100 cc.*	*1/hr.*	*mgm. per 100 cc.*	*liters*	*mgm. per minute*	*100 × cc. per minute*
1	1	0.288	143.26	1.878	6.44	2.60	5.25	0.527
	4	0.390	252.70	0.961	20.98	4.60	15.44	0.346
	5	0.401	1349.56	0.677	37.91	4.53	19.40	0.111
	6*	0.302	1461.05	0.885	0	3.64	0	0.235
	7	0.369	1570.87	0.821	10.42	4.09	5.83	0.190
2	2	0.219	291.57	0.919	8.95	3.58	4.91	0.330
	4	0.181	805.77	0.490	18.03	3.98	5.85	0.144
	5	0.196	864.37	0.416	32.68	4.24	9.61	0.098
	6	0.269	1444.43	0.454	55.39	4.71	19.75	0.087
3	1	0.347	359.84	0.846	27.35	4.03	15.53	0.221
	2	0.349	1388.91	0.636	68.15	3.80	27.41	0.053
4	1	0.272	1057.98	0.884	13.14	2.39	4.63	0.080
	2	0.270	1500.78	0.773	12.37	2.58	4.11	0.063
5	1	0.194	141.38	0.991	23.19	3.35	12.83	0.360
	2	0.325	793.22	0.783	19.85	3.71	9.60	0.159

* In this experiment the value of p cannot be distinguished from zero. Because the plasma data fall as in the other experiments, we believe that this value of p is due to the chance distribution of errors.

In view of these facts we consider equation 5 a satisfactory representation of the rate u, except at low values of the plasma concentration.

We have not attempted to give a mathematical expression to the whole curve of the rate u because of the difficulties encountered in the integration. But we have constructed this rate at low levels of the plasma concentration by fitting a polynomial in the time t to the terminal part of the curve of log ξ (fig. 3, upper right) and, from equation 4, determining u at a number of points. The details of this construction cannot be given here.[1]

[1] The computations used in this paper are quite laborious. For some of the applications, however, the values of the computed quantities can be obtained with sufficient approxima-

RESULTS. The foregoing analysis of the plasma curve answers the problems stated in the introduction.

1. The volume of distribution has been computed (equations 7 and 10). This volume varies between 2.39 and 4.71 liters of water (table 3). The mean value, 3.72 liters, with a standard error of 0.189, is significant. In per cent of the body weight, the volume varies between 18.0 and 35.4, with a mean of 24.9. Also in per cent of the body weight, the mean volume V of each dog is, in the order of table 3, 25.2, 24.8, 24.4, 21.6 and 25.2. In comparison with the large variation in both the quantity injected and the plasma concentration, the variation of the volume V is quite small (table 3). In fact, the coefficient of variation of the volume V is 0.190, a coefficient slightly larger than that of the body weight, 0.183, but considerably smaller than that of the excretion constant, 0.239.

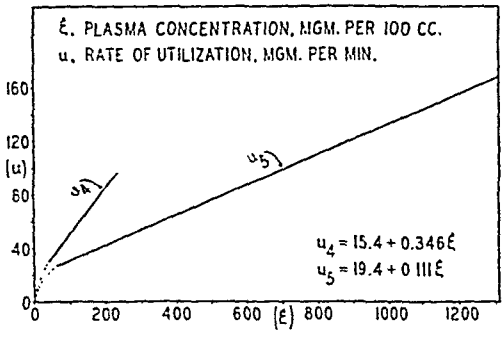

Fig. 4

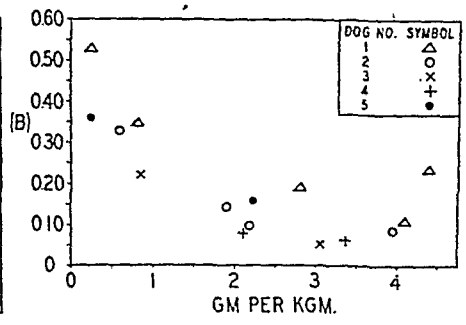

Fig. 5

Fig. 4. Relation of the rate of utilization of galactose to the plasma concentration. Because the lines are computed from experiments in which the plasma concentration falls continuously from the beginning of the experiment, the diagram is to be thought of as representing the change of the rate of utilization from the higher to the lower concentrations, that is, from the right to the left of the figure. The subscripts 4 and 5 in both the equations and the lines refer to the experiments 4 and 5 on dog 1, respectively. The lines u_4 and u_5 were computed by fitting equation 6. The interrupted curve from the left end of the line u_5 to zero was obtained from equation 4 by the construction outlined in the text.

Fig. 5. The utilization constant B depends on the quantity of galactose injected. In each dog, the larger the quantity injected, the smaller B. An exception occurs in experiment 6, dog 1, the experiment with the largest dose.

Therefore, the quantity of galactose present in the body at any time during the experiment can be determined by multiplying the concentration of galactose in the plasma by the volume of distribution.

2. The rate of utilization, that is, the rate at which galactose is removed from the plasma by or for conversion, has been determined in the presence of a simultaneous rate of excretion. From the initial plasma concentration down to

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tion by means of graphic analysis. After smooth curves have been drawn through the points representing the data on plasma concentration and rate of excretion, the quantities required in equation 4 for computing the rate of utilization can be estimated graphically. The only quantity which offers some difficulty is the slope of the tangent $\left(\frac{d\xi}{dt}\right)$ of the plasma curve. Adequate methods for drawing tangents are described in Daniels (1928).

values of this concentration that vary in different experiments, but are usually below 20 mgm. per 100 cc., the rate of utilization diminishes linearly with the plasma concentration (fig. 4). For values below this concentration, the rate of utilization diminishes rapidly and not linearly toward zero (fig. 4). The values of the constants a and B of the linear part (equation 5), computed from equations 7 to 10, are given in table 3.

Contrary to the slope A of the rate of excretion, the slope B of the linear part of the rate of utilization depends on the quantity injected. Thus, in figure 4, the slope of the line u_4 (10.65 grams injected) is three times as great as that of the line u_5 (57.74 grams injected). With the exception of experiment 6, dog 1, the plot of the slope B (table 3) and the dose in grams per kilo of body weight

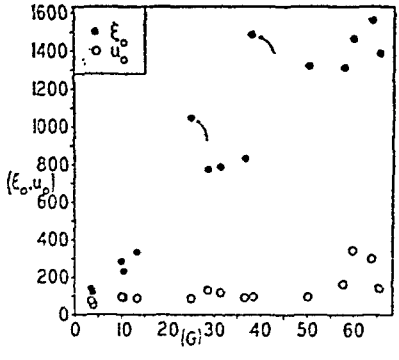

Fig. 6

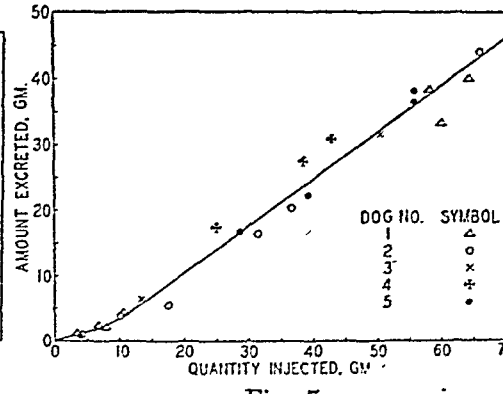

Fig. 7

Fig. 6. The initial plasma concentration ξ_0 is given in milligrams per 100 cc., the initial rate of utilization u_0 in milligrams per minute, and the quantity injected G in grams. In dogs of comparable size, the initial plasma concentration is proportional to the quantity injected (equation 10). The arrows point to the larger initial plasma concentrations of dog 3, the smallest dog. In experiment 6, dog 1 ($G = 59.7$), and in experiment 7, dog 1 ($G = 63.8$), the initial rates of utilization are exceptionally high.

Fig. 7. Relation of the amount of galactose excreted to the quantity injected. The curve following the mean position of the points rises at first with a slope not higher than 0.25, then, from about 4.0 to 10 grams, it bends upward, and, from a dose of about 10 grams up to the largest dose, 65.46, it rises linearly with a slope of 0.72.

(table 2) shows that, in each dog, the larger the quantity injected, the smaller the slope (fig. 5).

Although not established by these experiments, this systematic variation in the slope B points toward the existence of an upper limit to the initial rate of utilization. From the data of tables 2 and 3 it can be shown that the quantity injected, the initial plasma concentration, and the initial rate of excretion vary as 1:18, 1:13 and 1:25, respectively, but the initial rate of utilization varies only as 1:6. It is likely that beyond some dose, larger than any used here, there will be no further rise in the initial rate of utilization (fig. 6).

As a consequence of the effect of the dose on the rate of utilization, the proportion of excreted galactose is not constant. The amount excreted represents 22 to 30 per cent of the quantity given when the latter is less than 4.2 grams and 56

to 72 per cent when the quantity injected is more than 38 grams. This variation is illustrated in figure 7.[2]

DISCUSSION. Although no particular end product of conversion is being considered here, the calculated rate of utilization assigns an upper limit to the rate of conversion as a whole, for, if all the non-excreted galactose is converted into, let us say, glycogen, it is clear that the rate at which glycogen is formed from galactose cannot be greater than the rate at which galactose is removed from the plasma by or for the purpose of glycogenesis. If not all the non-excreted galactose is converted into glycogen, the proportion which is converted can be determined by comparing the excess of glycogen formed in a given time with the integral of the rate of utilization in the same time. By extending these investigations to other sugars, their efficiency for glycogen formation can be assessed.

If figure 4 is completed with the lines of the rates of utilization in all fifteen experiments, it will be seen that the path outlined by the initial values of the rate of utilization lies above the entire set of straight lines. This situation is true for the fall in plasma concentration after a rapid intravenous injection. If, on the other hand, the concentration in the plasma is made to rise slowly, what will be the course followed by the rate of utilization? Will it follow one of the lines already determined or will it follow the upper path outlined by the initial rates of utilization? If the concentration of galactose in the plasma rises at first and then falls, as in *oral* experiments, will the rate of utilization follow an upper path during the rise in concentration and a lower path during the fall in concentration? Until these points are settled for galactose and other glycogen-forming carbohydrates, it cannot be concluded that differences in glycogen formation are due to differences in either their glycogenetic efficiency or their rate of absorption from the intestine.

With respect to the variation in the proportion of excreted galactose, it should be recalled that, under the same experimental conditions, the proportion of excreted xylose is constant. This constancy is due to the fact that the rate of utilization of xylose is proportional to the plasma concentration and that the constant of proportionality is independent of the dose (Dominguez, Goldblatt and Pomerene, 1937). The metabolism of these two sugars is therefore quite different.

Finally, concerning the volume of distribution, there is no doubt that this volume represents a real partition of the body fluids even though the anatomical site of this partition is not specified. From the standpoint of the method used

[2] For the kinetics of elimination following a rapid intravenous injection, Teorell (1937) has proposed an equation identical in form to our equation for xylose (Dominguez, Goldblatt and Pomerene, 1937). Teorell obtains this equation in a theoretical way by assuming 1, Fick's diffusion law for the rate of diffusion into the tissues; 2, Fick's diffusion law for the rate of excretion, and 3, a chemical reaction of the first order for the rate of "inactivation". The second assumption is inadmissible. The third assumption is not sufficiently general. As shown here, the part of galactose which disappears from the plasma for conversion does not disappear at the rate of a monomolecular reaction.

here, this volume can be looked upon as a characteristic of any substance under investigation. This volume might be expected to be different with different substances. On the contrary, many substances, as dissimilar in chemical structure as in physiological properties, distribute themselves in volumes of quite comparable magnitude. Thus, sulfate, sucrose, chloride, xylose and thiocyanate—to mention only the substances recently compiled by Adolph (1943)—are distributed in volumes which do not differ much from the volume of distribution of galactose. Even creatinine, a substance which occupies eventually a volume two and a half times as large as that of galactose, enters first, and almost immediately, into a volume about equal to that of galactose (Dominguez, Goldblatt and Pomerene, 1935). These facts should be taken into consideration by physiologists interested in the anatomical interpretation of these volumes.

SUMMARY AND CONCLUSIONS

1. The disappearance of galactose from the plasma after a rapid intravenous injection has been studied in five dogs.

2. The data consist of the rate of excretion at consecutive intervals, the plasma concentration at selected times, and the endogenous blanks.

3. During and shortly after the injection, galactose diffuses into a volume of body fluids equivalent to about 25 per cent of the body weight. The concentration of galactose in this volume is in equilibrium with that of the plasma virtually from the beginning of the experiment. Hence, the amount of galactose present in the body at any time during the experiment can be calculated by multiplying the plasma concentration by the volume of distribution.

4. The subsequent fall in the concentration of galactose in the plasma is due only to renal excretion and chemical transformation. Diffusion takes no part in this fall.

5. The part excreted is lost at a rate (rate of excretion) which is proportional to the plasma concentration. The constant of proportionality is independent of the quantity injected.

6. The part transformed is removed from the plasma at a rate (rate of utilization) which is not proportional but is linearly related to the plasma concentration. The linear relation holds from the initial plasma concentration to concentrations of about 20 mgm. per 100 cc. or less.

7. The slope of the linear part of the rate of utilization depends on the quantity injected. In general, the larger the dose, the smaller the slope.

8. The initial rate of utilization does not rise in proportion to the quantity injected. To an 18-fold variation in the latter there corresponds only a 6-fold variation in the former. It is probable that the initial rate of utilization reaches a stationary value.

9. The plasma concentration can be represented by the equation

$$\xi = qe^{-kt} - p,$$

where ξ is the plasma concentration, t the time, and e the base of natural logarithms. The constants q, k, and p are defined in terms of (1) the volume

of distribution and (2) the constants relating the rates of excretion and utilization to the plasma concentration.

10. The equation of the plasma concentration is checked both by fitting the data and by calculating the amount excreted. The mean difference between the amount actually excreted and the amount calculated, expressed in per cent of the former, is about 3.5. The largest single error is found in the amount excreted in the first fifteen minutes after the injection.

11. From the relation of the rate of utilization to the dose it follows that the amount excreted is not proportional to the quantity injected. The amount excreted is less than 30 per cent of the quantity injected when the latter is less than 4.2 grams, and 56 to 72 per cent when the quantity injected is more than 38 grams.

12. The significance of the calculated rate of utilization in studies of the intermediary metabolism of carbohydrates is discussed.

We wish to thank Dr. A. J. Segal for making the figures used in this paper. We wish to thank also Prof. Harry Goldblatt and especially Prof. Charles F. Rehor, both of Western Reserve University, for their counsel in the preparation of the manuscript.

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CHANGES IN RIGHT AND LEFT CORONARY ARTERY INFLOW WITH CARDIAC NERVE STIMULATION[1,2]

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Received for publication February 17, 1944

Past investigations concerning the influence of the stellate ganglia and their associated nerves upon the flow of blood in the coronary arteries have yielded widely different results. According to some reports (1, 2, 3, 4, 5, 6), stimulation of these nerve structures causes an increase in coronary flow, while the findings of others (7) indicate the opposite, or a mixed effect (8). Examination of these reports reveals that a variety of experimental methods were used. In some instances, coronary inflow was measured only indirectly (coronary sinus outflow); in others, the data were obtained with unreliable instruments.[3] Except for the investigations of Morawitz and Zahn (1) and Greene (3, 4), the results were obtained in relatively abnormal preparations (fibrillating heart or heart-lung preparation). It is significant that in none of these studies was the coronary inflow measured by any method in a heart beating *in situ*.

While it is still not possible to quantitate coronary inflow under ideal conditions, the rotameter (12) and improved orifice meter (11, 13, 14) provide simple and accurate methods for determining coronary inflow in the anesthetized dog with much less disturbance than heretofore to the animal's nervous, hemodynamic and metabolic mechanisms. Both instruments can be used to measure mean rate of inflow; in addition, the orifice meter records a graphic picture of the phasic flow fluctuations in the main coronary arteries. Hence, the subject has been reinvestigated with the use of these instruments and a somewhat less abnormal preparation.

METHODS AND PROCEDURES. Dogs weighing from 10 to 29 kilos were given morphine preoperatively, anesthetized with either sodium pentobarbital, ether, or a combination of chloralose (100 mgm. per kilo) and urethane (50 mgm. per kilo) and then artificial respiration instituted. The right and/or left stellate ganglia together with their cardiac branches[4] (nerves) were exposed by resection of part of the second rib. The heart was exposed by removal of part of the fifth left rib and the coronary artery to be studied isolated with care for subsequent cannulation. After injection of an anticoagulant (usually a combination of

[1] The expense of this investigation was defrayed to a large extent by a grant from the Commonwealth Fund.

[2] The authors with to express their appreciation to Dr. A. Rotta (now in the department of physiology, Univ. of Peru, Lima) and to Dr. W. R. Pritchard of this department for their assistance in some of the earlier experiments.

[3] Errors in the thermostromuhr method (9, 10).

[4] For convenience, the term "cardiac branches" or "cardiac nerves" is used to denote all nerve fibres arising from a stellate ganglion exclusive of the rami of the connecting spinal nerves.

heparin and chlorozol or pontamine fast pink), a trocar was inserted into the common carotid or subclavian artery,[5] to the level of the aortic valves and blood led from it through a lead tube, either to a rotameter or to an orifice meter. The peripheral end of the meter was connected to the peripheral cut end of the coronary artery being studied. Aortic blood pressure was determined by a Gregg pressure manometer (14, 15, 16) and recorded optically together with the phasic or mean rate of flow. In some experiments, the stellate ganglia were stimulated after their isolation from the sympathetic chain (accomplished by sectioning above and below the ganglia). In others, the cardiac nerve fibres arising from the stellate ganglia were stimulated before and after section from their ganglia. In all experiments, the nerve structures were stimulated by a Harvard inductorium and continuous readings made before, during and after such stimulation. In some dogs the hearts were driven by an artificial pacemaker attached to the right auricle. In some cases, the blood pressure was kept at the control level by the adjustment of a clamp applied to the aorta just above the diaphragm. In most instances, flow measurements were made within 30 to 60 minutes after anesthetization of the dog.

RESULTS. If injury to nerve structures, incident to their isolation, was minimal, stimulation of the stellate ganglia or their cardiac fibres caused a large increase in coronary inflow. The flow increase usually began a few seconds after the start of the stimulation and lasted from a few seconds to several minutes after stimulation had ceased. In general, the magnitude of the flow increase was less, the weaker the intensity of the stimulation, the shorter the time interval between successive stimulations and the later in the experiment the stimulus was applied.

A. *Changes in mean rate of inflow.* Augmentation of flow in either main branch of the left coronary artery occurred in all of 21 experiments in which either or both stellate ganglia and their cardiac branches were stimulated before and after section from the sympathetic chain. In no instance was the coronary inflow found to decrease. However, in several experiments, nerve stimulation elicited either a very small increase or no change in coronary flow. In some experiments stimulation was accompanied by an appreciable augmentation of heart rate or blood pressure; in other cases, the heart rate and blood pressure were not noticeably affected, while in still others, a concomitant rise in blood pressure was artificially compensated and kept at the control level during nerve stimulation.

The experiment illustrated in figure 1B is typical of that group in which, during nerve stimulation, no attempt was made to control the blood pressure or heart rate and the blood pressure rose moderately. With a control flow of 23 cc. per minute into the left circumflex artery and a mean blood pressure of 80 mm. Hg,

[5] In many experiments, to obviate the criticism that the length of the coronary blood flow circuit might cause excessive damping of the flow pattern or appreciable reduction in coronary flow, the chest plate was removed and blood led (by a cannula inserted into the left subclavian artery to the level of the aortic valves) to the recording instruments, and thence to the coronary artery. In actual tests in the same dog, the patterns and flows obtained alternately in the long and short circuits were not measurably different.

stimulation of the cardiac fibres from the left stellate ganglion (after section from their ganglion) for two minutes increased the flow greatly to 72 cc. per minute (213 per cent increase) where it remained as long as the stimulation continued. Upon removal of the stimulus, the flow decreased and after nine minutes was still

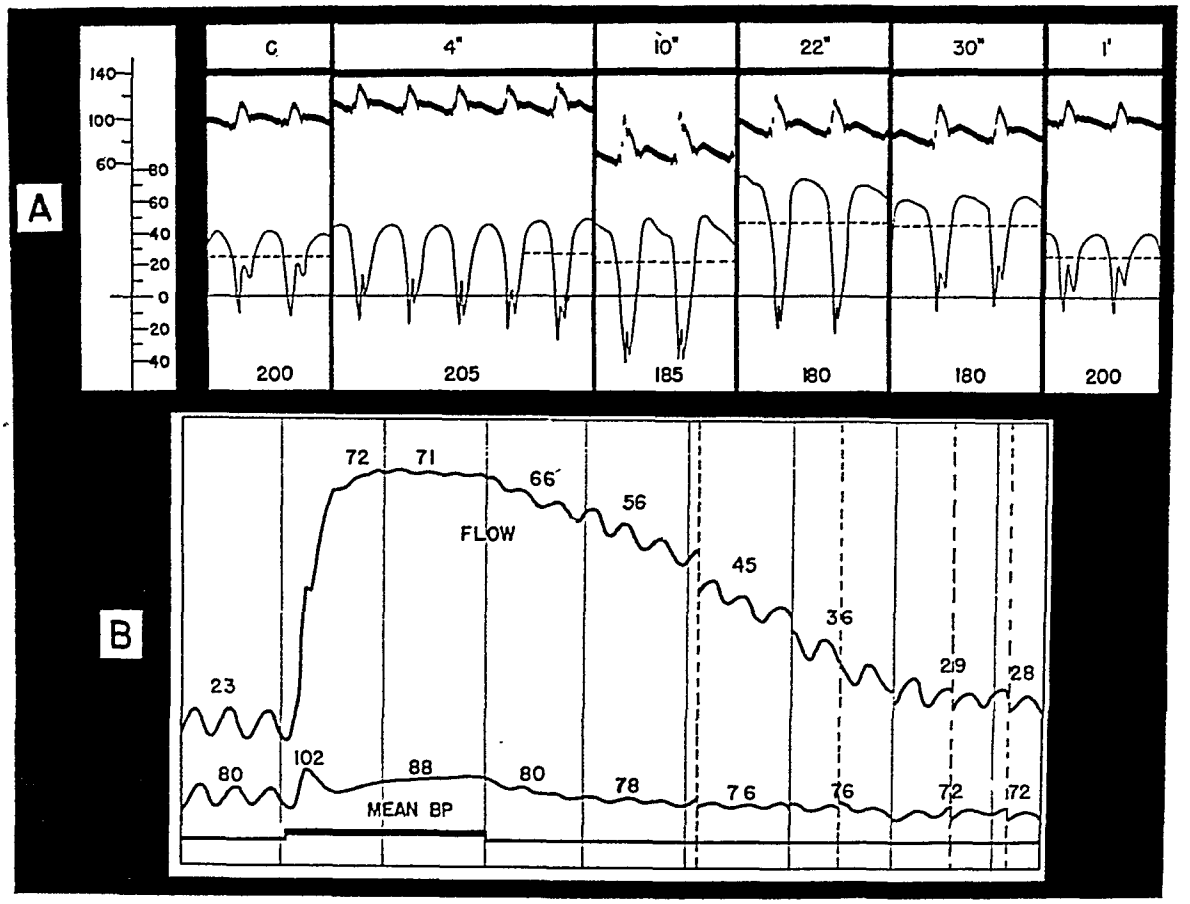

Fig. 1A. Reproductions of a series of original pressure (upper) curves and rectified reconstructions (lower) of original velocity flow curves recorded in the descendens branch of the left coronary artery following stimulation of the left cardiac nerves. Dog weight 12.2 kgm. Ordinate scales—pressure in millimeters of mercury on left, flow in cubic centimeters per minute on right. Continuous horizontal line indicates zero flow level. Interrupted line indicates mean flow level for each record segment. Numbers on each record segment are, at top, time elapsed following beginning of nerve stimulation and, at bottom, heart rate. *C*, control record.

Fig. 1B. Tracing of original record showing augmentation in mean blood flow as measured by a rotameter in the circumflex branch of the left coronary artery following a 2 minute stimulation of left cardiac nerves. Numbers on curves, cubic centimeters per minute and millimeters Hg. Solid vertical intercepts, one minute intervals. At broken vertical intercepts, recording stopped for ½ minute intervals.

somewhat greater (28 cc. per min.) than the control flow. During stimulation, the mean blood pressure underwent a transitory rise from 80 to 102 mm. Hg, and then almost immediately (½ min.) fell to approximately the control value, and during the recovery period to somewhat below the control value. The heart rate (104 per min.) showed no significant change.

Examples of the other types of response indicated above are given in table 1. Similar, but generally smaller, increases in right coronary inflow were observed (8 out of 9 expts.) during comparable nerve stimulation (cf. table 1). In one experiment, no change in coronary flow was observed.

TABLE 1

Effect of cardiac nerve stimulation on coronary inflow

EXPERIMENT	HEART RATE		AORTIC BLOOD PRESS.		CORONARY INFLOW CC. PER MINUTE		CONDITION
	Before	During	Before	During	Before	During	
Left coronary artery							
1	190	180	113/96	118/85	27	45	Left cardiac nerves cut from stellate ganglion and stimulated. Flow measured in left descendens. Blood pressure not compensated
2	108	150	95	90	18	39	Same as expt. 1
3	125	135	67	72	14	30	Left cardiac nerves cut from stellate ganglia and the inferior fibres stimulated. Flow measured in left circumflex. Blood pressure not compensated
4	140	145	108	108	16	32	Same as expt. 3 but blood pressure compensated
5	120	120	110	110	17	45	Same as expt. 4
6	146	143	132	136	28	46	Same as expt. 3
7	104	104	80	91	23	72	Same as expt. 3
8	132	138	110	108	16	32	Same as expt. 4
Right coronary artery							
1			126	120	27	32	Right cardiac nerves cut from stellate ganglion and stimulated. Blood pressure not compensated
2	146	168	76	74	10	15	Both right and left cardiac nerves cut from stellate ganglia and stimulated. Blood pressure compensated
3	140	144	86	90	23	44	Right cardiac nerves stimulated (not cut). Blood pressure not compensated
4	152	152	95	97	25	37	Left cardiac nerves stimulated (not cut). Blood pressure compensated
5	162	162	90/68	99/68	33	33	Left inferior cardiac fibres stimulated (not cut). Blood pressure not compensated

B. *Changes in flow pattern.* As with the rotameter, coronary inflow recorded by the orifice meter increased in both right and left coronary arteries with cardiac nerve stimulation. Phasic changes in the left coronary inflow pattern in one experiment are illustrated in figure 1A. Blood pressure and heart rate were not artificially compensated. In the control segment, a sizable backflow was pres-

ent during early systole and the diastolic rate of flow was considerably greater than that during systole. Within four seconds after the beginning of nerve stimulation, the mean blood pressure rose mildly (18 mm. Hg), the systolic rise in pressure becoming more abrupt and peaked. In the flow pattern, the diastolic flow increased progressively while the systolic flow decreased rapidly from beat to beat and became almost entirely backflow. Within ten seconds, the blood pressure spontaneously fell to a subcontrol level, but the pressure curve retained its sharp systolic rise. Further depression in systolic flow occurred with an accompanying small reduction in mean rate of flow. Within 22 seconds, the blood pressure spontaneously rose to approach the control level, the systolic reduction in flow became less, while the diastolic and mean rates of inflow increased greatly. These changes were well maintained to 40 seconds (end of stimulation). At one minute, the pressure and flow curves returned to the control levels.

The above illustrates the changes in phasic flow distribution and blood pressure which frequently accompany an increase in mean rate of inflow into the left coronary artery with nerve stimulation. While, in all experiments, the diastolic flow increased and the systolic flow decreased, in other particulars the response varied. Frequently the blood pressure did not fall and the flow and pattern responses were similar to the sequence in the 4 second and 22 second records. In other experiments, artificial compensation of the blood pressure to the control level gave results not discernibly different from those shown in the 22 second record of figure 1A.

Flow patterns recorded in the right coronary artery showed directional changes which were similar to those recorded in the left coronary artery, i.e., flow during systole either decreased or remained the same while the diastolic flow increased considerably. In some instances, a sustained phasic redistribution of flow was observed without an appreciable increase in mean rate of inflow, the diastolic increase in flow being compensated by the flow reduction during systole. (This also occurred in the left coronary artery but was transient in appearance (cf. 4 second record, fig. 1A).)

Discussion. Stimulation of the stellate ganglia and/or their cardiac branches in the anesthetized, open-chest dog is found to increase the flow in both right and left coronary arteries. The increased coronary inflow occurs with or without spontaneous elevation of blood pressure and heart rate, or when the aortic blood pressure is artificially controlled and compensated to the control level. This indicates that the factors of heart rate and blood pressure are not indispensable to the natural mechanism through which the increase in flow is mediated.

The observed increase of coronary inflow is regarded as evidence of coronary dilatation. The mechanism by which such nerve stimulation causes dilatation of the coronary vessels or otherwise increases coronary inflow cannot be identified in these experiments.

The extensive alterations of the coronary inflow patterns observed with nerve stimulation are related to the influence of many complex and interdependent factors. The pattern changes are regarded as the summated effects of alteration

in some or all of the following interrelated variables; vasomotor state, vessel elasticity, volume-elasticity coefficient, frictional resistance (viscosity effects), magnitude and rate of change of aortic pressure, extravascular compression (support) and rate of flow. While others (17, 18) hold the view that changes in vasomotor state and extravascular support can be deduced from alterations in these curves, we feel our present knowledge does not justify such a procedure. The patterns are presented with the hope that subsequent studies will permit an adequate interpretative analysis of them.

Attention is directed to the fact that the nerves studied were stimulated by artificial means at a rate and intensity and under conditions which may or may not approximate those naturally occurring in the undisturbed nerve fibres. Hence, it is not known whether the naturally occurring nerve action currents would cause similar changes in blood pressures, heart rate and coronary inflow.

SUMMARY

The effects of stimulation of the stellate ganglia and their cardiac branches on coronary inflow in the anesthetized, open-chest dog have been studied with the use of the rotameter (for recording mean rate of inflow) and the orifice meter (for recording phasic flow).

Stimulation of these structures usually causes a considerable and sustained augmentation of left coronary inflow and a somewhat smaller increase in right coronary inflow. In no instance was a sustained reduction in flow observed.

The increase in mean rate of flow is the resultant of a decreased inflow during systole and a proportionately larger increase in flow during diastole. Occasionally, the phasic redistribution of flow throughout the cycle is such that the net flow increase is quite small.

The augmentation of coronary inflow is frequently accompanied by simultaneous elevation of aortic blood pressure and/or heart rate. However, observations that the inflow increases when these variables either do not increase spontaneously or are artificially kept at the control level, indicate that these factors alone are not an indispensable part of the mechanism by which coronary inflow is increased.

The augmentation of coronary inflow is regarded as an indication of coronary vessel dilatation. The mechanism remains to be determined.

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THE EFFECT OF POSTERIOR HYPOPHYSECTOMY ON RENAL HYPERTENSION[1]

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Received for publication January 15, 1944

The very existence of the neurohypophyseal pressor substance, and further, the recent demonstration (1, 2) that it may be reflexly secreted in effective amounts, points to the necessity of determining whether this substance plays any rôle in the mechanism of the various naturally occurring and experimental forms of hypertension.

Direct attempts to study the possible relationship between the pituitary body and renal hypertension (3, 4, 5) have led to inconclusive results. This may be due in part to the failure in some instances to separate completely the effects of the anterior and posterior lobes of the gland. Further attempts to resolve the question have not appeared, perhaps partially because the present concepts of renal hypertension leave less room for a posterior pituitary mechanism to play any essential rôle. The present report constitutes an extension of the work preliminarily reported by the authors (6).

METHODS. Adult rats of both sexes, maintained on a complete stock diet, were used throughout. Arterial hypertension was produced by partial ligation of the left renal artery, following the technique of Wilson and Byrom (7), except in 3 rats in which silk bags were loosely applied around both kidneys, and 1 in which collodion was applied to the kidney. Our past experience has indicated that approximately half of all rats with attempted partial ligation of one renal artery develop hypertension. The failures are due to complete ligation, too loose a tie, or to a failure to place the tie proximal to the point where the renal artery branches.

Systolic blood pressure was measured at frequent intervals (from one to four times a week) by the tail plethysmograph method of Williams, Harrison and Grollman (8) on warmed, unanesthetized rats. After the initial period of training, the average systolic tail pressure of normal young adult rats was found to be 104 mm. Hg with a standard deviation of $\pm$ 5 mm. We have found no instances of sustained spontaneous hypertension in studying over 500 normal rats. Persistent mean pressures over 130 mm. occurred in our series only after experimental interference.

Posterior or complete hypophysectomy was performed either before or after the renal surgery. A control group of ten animals underwent a sham operation in which the posterior hypophysis was exposed and gently pulled. The intake

[1] Supported by grants from the John and Mary Markle Foundation, the Board of Research of the University of California, and the Christine Breon Fund.

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of water per 24 hours at standard environmental temperature of 28.5°C. was determined for a period of 5 days beginning 2 or 3 weeks after the operation. In a series of 13 unoperated rats of the same age as the experimental group the water intake ranged from 6.7 to 17.9 (ave. 11.6) cc. per 100 grams body weight per 24 hours, and this series was used to judge the effectiveness of the posthypophysectomy. The presence of a normal amount of functioning anterior lobe was based on the weight and microscopic appearance of the gonads, thyroid and adrenal cortex; gain or loss of body weight; basal metabolic rate; and serial sections of the sella turcica. Thirteen hypertensive rats developed deficient or absent anterior lobe function, and this group is considered separately.

RESULTS. *Renal surgery after posterior hypophysectomy.* Sixteen rats which had persistent diabetes insipidus observed over a period of 7 to 11 months after posterior hypophysectomy, were subjected to a partial ligation of the left renal artery. The results are given in table 1. Ten rats, or 62 per cent of this group, developed hypertension as evidenced by a sustained increase of more than 25 per cent above the normal blood pressure. The percentage of success in producing hypertension by this method, as well as the duration and distribution of severity of the hypertension, closely parallel our experiences with normal rats. The presence of diabetes insipidus appeared to exert no influence upon either the production or maintenance of renal hypertension.

Posterior hypophysectomy after renal surgery. The posterior pituitary lobe was successfully removed from 15 additional rats which had had hypertension established for periods varying from 4 to 40 weeks after partial ligation of the left renal artery. Care was taken to see that the level of hypertension was sufficiently stabilized within a narrow range of spontaneous fluctuation before the pituitary operation. All rats showing any deficiency of anterior pituitary function were discarded from this group. The results are summarized in table 2. One rat showed a marked increase in the severity of hypertension, and 9 did not change significantly (i.e., more than ± 25 mm. Hg). Five showed a decrease greater than 25 mm. Hg in blood pressure level. This decrease occurred slowly, during the first month postoperatively. Five of these rats were then given daily injections of Pitressin Tannate in oil[3] for 12 days in amounts (0.5 unit) which proved to be sufficient to correct the diabetes insipidus. This did not cause an increase in blood pressure as might have been expected if the removal of the source of posthypophyseal pressor substances had been a major determinant of the fall of blood pressure noted in some of these rats. The rise or decline of blood pressure after removal of the posterior lobe did not seem to be related to the preoperative duration of the hypertension.

Complete hypophysectomy after renal surgery. From 12 hypertensive animals (table 3) the whole pituitary body was removed as evidenced by loss in body weight, atrophy of gonads, adrenal glands and thyroid, or lowered oxygen consumption, together with absence of diabetes insipidus. All of these rats showed a lowering of blood pressure. The average fall was 38 mm. Hg in the first week

[3] Supplied by Dr. Oliver Kamm of Parke Davis Laboratories.

and a further decrease of 12 mm. Hg in the second week after hypophysectomy, but the blood pressure usually remained definitely above normal values.

Sham hypophysectomy after renal tie. Ten rats, suitably hypertensive, underwent control operations in order to study the influence of the surgery alone. The operation consisted of a sham hypophysectomy in which the pituitary was exposed but not removed. Four rats demonstrated an increased blood pressure

TABLE 1

The production of hypertension in rats after removal of the posterior pituitary

Water intake (cc./100 gm. BW/24 hr.)	24.5	*17.5	18.5	19.6	*15.9	*14.9	20.6	21.4	21.0	*16.8	19.4	28.3	21.5	21.0	18.5	37.0
B. P. after renal surgery (mm. Hg)	103	105	106	108	108	112	132	136	140	148	151	151	157	158	184	196

* These four rats had shown water intakes indicating undoubted posterior lobe insufficiency, but their water intake had dropped somewhat before the renal surgery.

TABLE 2

The effect of posterior hypophysectomy upon the blood pressure of rats with experimental hypertension

																Av.
Water intake (cc./100 gm. BW/24 hrs.)	50.2	23.2	23.5	22.0	42.0	23.1	22.8	28.6	22.3	17.3	19.3	29.2	38.9	18.4	23.7	27.0
B. P. before posthypophysectomy (mm. Hg)	146	191	139	199	154	146	138	168	191	131*	147*	175*	186	217*	195*	168
B. P. after posthypophysectomy (mm. Hg)	218	212	152	211	166	157	129	157	173	110	118	140	135	145	114	156

The blood pressures represent the means of all readings during a 4 week period before and a similar period after removal of the neurohypophysis (excluding the first week postoperatively.) A change exceeding 25 mm. Hg is considered significant. The data are arranged in order of change of blood pressure from +72 to −81.

* Pitressin Tannate in oil administered without effect on blood pressure (see text).

TABLE 3

The effect of complete hypophysectomy upon the blood pressure of rats with experimental renal hypertension

													Av.
Mean B.P. before hypophysectomy (mm. Hg)	200	200	180	240	175	160	170	200	210	165	180	195	189
Mean B.P. 2nd week after hypophysectomy (mm. Hg)	180	165	160	160	140	140	130	125	125	120	120	110	139

(39–90 mm.), five did not change and one had a slightly (20 mm.) decreased blood pressure. We attribute the increases of blood pressure simply to a progression of the renal hypertension uninfluenced by intracranial manipulation.

Discussion. The finding that rats with experimental diabetes insipidus may be readily made hypertensive by partial ligation of the renal artery and that renal hypertensive rats may have the posterior lobe removed with no consistent change in their blood pressure leads to the definite conclusion that this lobe plays no essential part in the genesis and maintenance of experimental renal hypertension. This does not conflict with the findings of others; for in all reports in which pitui-

tary surgery has interfered with the mechanism of renal hypertension there has been deliberate damage to the anterior lobe.

The significant decline in blood pressure which occurred in some instances during the first month after removal of the posterior lobe is not due to the removal of an essential pressor hormone, for it was observed in only one-third of the animals. Moreover, replacement of pitressin in doses adequate to control the diabetes insipidus was without effect on the blood pressure. Similar observations on dogs following section of the neurohypophyseal stalk were noted by Sattler and Ingram (5). It might be claimed that the difference of minimal dosage for the vasopressor and antidiuretic effects would invalidate this argument, but on the other hand if the animals which did maintain their hypertension with diabetes insipidus had an amount of hormone inadequate for water control how much less adequate would it be for maintenance of hypertension!

It is most likely that the invariable fall of blood pressure after complete hypophysectomy is due to removal of the anterior hypophysis. Further experimental evidence for this is reported in another paper (9).

SUMMARY

Partial ligation of one renal artery is as likely to produce hypertension in rats after posterior hypophysectomy as in normal animals. Ablation of the posterior lobe alone does not consistently lower the blood pressure of hypertensive rats. Pitressin in amounts sufficient to relieve the diabetes insipidus does not restore the pressure of those rats in which a decline was observed. Sham hypophysectomy does not alter the course of the hypertension.

By contrast, complete hypophysectomy lowers the blood pressure of hypertensive rats.

The posterior lobe is not necessary for the initiation or maintenance of experimental renal hypertension.

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RESTORATION OF RENAL HYPERTENSION IN HYPOPHYSECTOMIZED RATS BY THE ADMINISTRATION OF ADRENOCORTICOTROPIC HORMONE

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Received for publication January 15, 1944

The influence of the hypophysis on blood pressure was recognized by Cushing (1) and by Holmes (2) in their clinical observations of cases of pituitary tumors. They called attention to the hypotension associated with pituitary insufficiency in patients with craniopharyngiomata and chromophobe adenomata. Recently Escamilla and Lisser (3) have published an extensive survey of the reported cases of Simmonds' disease. They found in a series of 101 verified cases of pituitary cachexia, that 38 per cent of the cases had systolic blood pressure readings below 90 mm. Hg.

Page and Sweet (4) first showed that removal of the pituitary of hypertensive dogs reduced the level of the blood pressure markedly; however, a further increase in constriction of the renal artery produced a rise in blood pressure though less marked and not sustained. The observations of Goldblatt et al. (5) are less conclusive in this regard. In their studies, complete hypophysectomy did not prevent the development of experimental renal hypertension and they concluded, therefore, that the hypophysis played no significant part in the pathogenesis of experimental renal hypertension. This latter view is not entirely consistent with the experimental proof of a relationship between the adrenal cortex and the hypophysis.

It has been well established by several investigators (6, 7, 8, 9, 10) that the adrenal cortex is a necessary part of the mechanism by which renal hypertension is produced and sustained. Since the adrenal cortex is one of the target glands under the influence of the anterior hypophysis it is to be expected that hypophysectomy which is always followed by a decrease in the function of the adrenal cortex would lower the blood pressure of renal hypertension. The findings of Page and Sweet (4) bear this out. The data presented in this paper carry the evidence another step toward this conclusion.

EXPERIMENTAL. The methods employed in this study have been described in a previous communication (11). Male rats approximately 2 months of age were made hypertensive by a partial ligation of the left renal artery. After the blood pressure had reached a hypertensive plateau, which occurred usually in 6 to 10 weeks, the animals were hypophysectomized. The blood pressure fell in every case, the amount of fall depending upon the length of time in which the

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animal had been hypertensive.[3] In very few instances did the blood pressure fall to below 120 mm. Hg which is probably about the upper limit of normal values (table 1). Before hypophysectomy, the average blood pressure reading in the group of hypertensive rats in this table was 181 mm. Hg. During the second week after hypophysectomy the mean blood pressure for the group had fallen to 137 mm. Hg. The average drop was 44 mm. Hg.

Two weeks after hypophysectomy the animals were injected with adrenocorticotropic hormone prepared by the method of Li, Evans and Simpson (13). This

TABLE 1

The effect of hypophysectomy and of replacement therapy with adrenocorticotropic and lactogenic hormones on renal hypertension

BEFORE HYPOPHYSECTOMY	AFTER HYPOPHYSECTOMY	ADRENOCORTICOTROPIC FOR 10 DAYS		
B.P. (1)	B.P. (2)	Dose (3)	B.P. (4)	Period of decline (5)
		mgm./day		*days*
205	124	3.0	198	10
180	160	3.0	205	11
205	162	3.0	200	11
162	138	3.0	175	11
165	120	1.0	184	7
188	120	1.0	176	5
235	165	1.0	215	4
175	140	0.1	165	3
170	130	0.1	143	5
		LACTOGENIC HORMONE FOR 10 DAYS		
151	129	3.0	127	
160	129	3.0	132	
175	123	3.0	119	

(1) Average of 6 readings taken during the 2 weeks before hypophysectomy. Hypertension established 3–48 weeks before hypophysectomy.

(2) Average of 4 readings taken during the 2nd week after hypophysectomy.

(3) The daily dose was divided and given in 3 injections.

(4) Blood pressure on last day of injection.

(5) Number of days after the last injection before blood pressure returned to pre-injection level.

preparation has been found to be free of other anterior hypophyseal hormones by biological tests. Furthermore, from electrophoretic, sedimentation and solubility experiments, the preparation appears to be a single substance. The hormone was administered in dose levels of 0.1 mgm., 1.0 mgm. and 3.0 mgm. daily for 10 days. Each daily dose was divided and given in three intraperitoneal injections. By this method of administration 0.2 mgm. of the pure hormone

[3] Compare the findings of Page, Patton and Ogden (12) that removal of the affected kidney in rats hypertensive from unilateral renal artery obstruction lowered blood pressure in proportion to the duration of the hypertension.

daily is sufficient to maintain the preoperative adrenal weight in male rats hypophysectomized at 40 days of age.

Three of the animals were injected with a lactogenic hormone under similar conditions as above, using 3.0 mgm. intraperitoneally daily for 10 days. The lactogenic hormone was prepared by the method of Li, Simpson and Evans (14) and had been shown to be chemically pure by electrophoretic, solubility and diffusion tests. The activity was 25 to 30 IU/per mgm. These three animals serve to control the possibility of our observations being influenced by substances from the anterior pituitary other than adrenocorticotropic hormone for in spite of the fact that the lactogenic hormone is closely allied chemically, indeed difficult to separate from the adrenocorticotropic fraction, it had no influence upon the blood pressure. This illustrates the specificity of the effect observed.

The data are presented in table 1. It will be seen that the adrenocorticotropic hormone when given in amounts as low as 0.1 mgm. daily causes a significant rise in blood pressure; a dose of 1.0 mgm. daily seems to give complete functional replacement as judged by the return of the blood pressure to the pre-hypophysectomy level. A higher dose of 3 mgm. daily had a similar effect. It did not cause the blood pressure to rise above the hypertensive level present before hypophysectomy. However, the hypertension was sustained for a longer period with the larger dose. The lactogenic hormone had no effect upon the blood pressure level.

Discussion. In addition to clarifying the relationship between the pituitary body and experimental hypertension, these findings add to the growing body of evidence that the adrenal cortex plays an important and essential part in the renal pressor mechanism. It appears increasingly evident that this mechanism, so far from being a laboratory or pathological curiosity, responds rapidly and regularly to moderate physiological stimuli and is probably normally helping to maintain the cardiovascular system in a state of physiological homeostasis; and it may well be that we should look to the renal pressor system for the clue to understanding the mechanism of endocrine influence upon blood pressure.

SUMMARY

Hypophysectomy causes a fall of the blood pressure of rats with renal hypertension. The blood pressure does not fall to a normal level except in those animals in which the hypertension has been present less than one month. A purified adrenocorticotropic hormone will restore the renal hypertension to the pre-hypophysectomy level. Lactogenic hormone in pure form does not show this effect.

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AUDIO FREQUENCY LOCALIZATION IN THE ACOUSTIC CORTEX OF THE DOG[1]

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Received for publication January 13, 1944

The areas of the cerebral cortex to which the acoustic system projects have not been reported in detail in the dog, although Ferrier (7) pointed out many years ago that the middle ectosylvian gyrus of this animal is the cortical zone for hearing. Woolsey and Walzl (21) recently have shown that the cochlea is represented in the cerebral cortex of the cat by electrical stimulation of the bony spiral. Preliminary reports of audio frequency localization in various species also have appeared in recent literature. Studies now in progress on the acoustic association connections of the dog made necessary a more exact investigation of the areas receptive to tones in this species. Experiments to this end, both by audio and electrical stimulation, were therefore undertaken, the results of which are presented in the following pages.

METHODS. The animals were deeply anesthetized with nembutal or with a 10 per cent urethane solution of Dial-Ciba.[2] Usual surgical supportive measures maintained the condition of the animal. The ectosylvian and sylvian gyri were exposed with a circular cranial opening, which was liberally surrounded with cotton soaked in Ringer's solution. A sheet lead ring 9 cm. outside diameter and 6 cm. inside diameter was embedded in the cotton, concentric to the opening. The ring was grounded and through a cotton bridge soaked in Ringer's solution, served as the indifferent electrode (4). The active electrode was a cotton wick moistened with Ringer's solution which was moved over the cortex in 1 to 2 mm. co-ordinate divisions. The indifferent and active electrodes were led through 1.0 mfd. condensers to the input of a 5 stage differentially balanced push-pull amplifier whose output was fed into a cathode ray tube.

Acoustic stimulation. The audio frequency signal was generated by an oscillator (6), whose output was fed in another room into the grids of a 79 tube (19). Direct current impulses synchronized with the sweep circuit of the oscillograph were fed into the grid circuit of a 6F6 power amplifier tube, operating below the plate current cutoff point. The flow of plate current in the 6F6 tube, caused by these pulses, activated a relay which eliminated a high negative grid bias voltage in the 79 tube, allowing the audio signal to flow into a baffled loudspeaker. An attenuator was placed in the input circuit of the 79 tube to reduce the minimum output of the oscillator. The output of the oscillator was considered constant over the range of 100 to 8000 cps.

For uni-lateral stimulation, after destruction of the cochlea of the opposite

[1] Aided by a grant from the Research Council of the Oregon State System of Higher Education.

[2] The Dial-Ciba was kindly placed at the author's disposal by the Ciba Company.

ear, a loudspeaker was placed directly opposite the animal's ear at a distance of about 1 meter for the contra-lateral and ½ meter for the ipsi-lateral side. The initial click free wave front from the loudspeaker constituted the physiological stimulus. Because of non-uniformity of any loudspeaker response (16), modification of sound by conduction, and lack of suitable microphonic equipment, no calibrations were made in terms of decibels. In conformity with neurophysiological methods, the stimulus value is denoted as threshold, maximal, sub-maximal and supra-maximal for the cortical response. The cortical focus of a frequency was determined by using a minimum threshold stimulus.

A microphone situated at the level of the oval window was connected through another amplifier into a second cathode ray tube.

Electrical stimulation. After exposure of the cochlea, stimulating electrodes, consisting of the tips of a pair of no. 40 enamel covered copper wires, were placed on the bony lamina 0.5 to 0.7 mm. apart. The shock was a condenser discharge of a magnitude causing a threshold cortical response.

Twelve dogs were used in the present study; 3 for ipsi-lateral, 3 for contra-lateral, and 1 for both contra- and ipsi-lateral audio frequency stimulation; 4 for ipsi-lateral and 1 for contra-lateral electrical stimulation of the cochlea.

RESULTS. Responses from the contra-lateral cortex possessed a lower threshold than those of the ipsi-lateral side. On both sides the usual initial response for the focus of a frequency was surface positive (fig. 5). With supra-maximal stimulus values, small surface positive responses were recorded for a distance of several millimeters from the suprasylvian and the dorsal portion of the coronal gyri. Initial negativity was encountered in all experiments in various parts of the cortex, but was consistently present along the middle and anterior ectosylvian gyri bordering upon the suprasylvian sulcus (fig. 3). The negativity was obtained to all audio frequencies, to the electrical shock, and has been observed to click stimulation. One point was responsive to a wide range of frequencies.

Contra-lateral side. Figure 2 illustrates the foci of response for various frequencies of the dorsal area (fig. 1) along the middle ectosylvian gyrus, high frequencies anteriorly and low frequencies posteriorly. Thresholds varied with frequency—decreasing toward the high. The outlines represent the responsive area for each frequency studied at its own threshold value. In general, responses were largest near the centers of the foci, with diminishing size and variation of latencies as the periphery was approached. The foci were arranged linearly about 2 mm. apart, with neighboring frequencies related to each other as octaves. Responses to 20000 cycles, the maximum output of the oscillator, were found anterior to and overlapping considerably with the area for 16000. A moderate concentration of foci was observed for frequencies 100 to 400.

Ventrally, on the anterior ectosylvian gyrus, responses which required a stimulus supra-maximal for the dorsal area were obtained to frequencies 100 to 800, but with smaller intervals. Frequencies 4000 to 16000 elicited responses from the posterior ectosylvian gyrus with a similar pattern; 800 and 4000 appeared to spread toward the ectosylvian sulcus. Responses to 1600 and 2000 were recorded from these areas into the region of the sulcus and the sylvian gyrus.

Discrete foci from the sylvian gyrus were obtained infrequently. Under deep anesthesia (fig. 2) responses to 4000 were limited to the posterior ectosylvian gyrus, and responses to 800 to the anterior ectosylvian gyrus, spreading slightly upon the sylvian gyrus. Localized points of response to 1600, 2000 were found upon the sylvian and posterior ectosylvian gyri. Under lighter anesthesia, the areas for 400 and 800 spread upon the anterior portion of the sylvian gyrus, 4000 spread from the posterior ectosylvian gyrus over the sylvian gyrus, and 1600, 2000 extended from the margins of the area responsive to 16000 across the sylvian gyrus to include a large portion of the area for 800. The responses to 800, 1600, 2000 and 4000 upon the sylvian gyrus were small, 50–100 microvolts. In the latter experiment the sylvian gyrus was removed along the ectosylvian sulcus and the adjacent walls of the ectosylvian gyrus were explored. Responses were

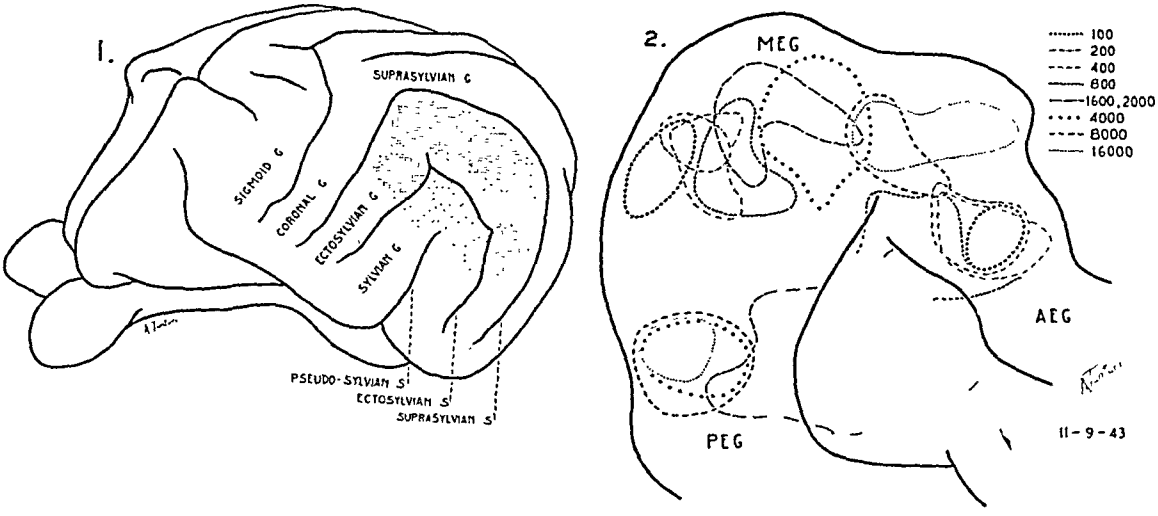


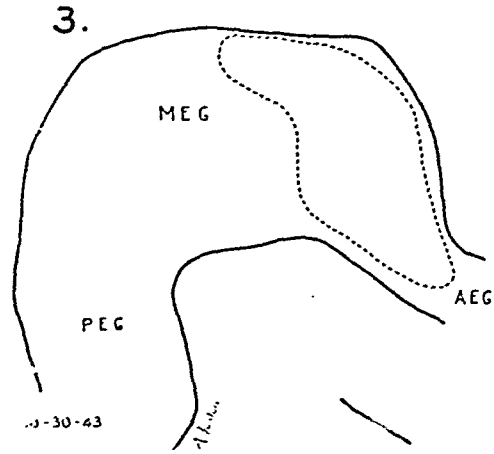
Fig. 1. Dorsal and ventral areas in the acoustic cortex of the dog in which localization to audio frequencies was demonstrated.

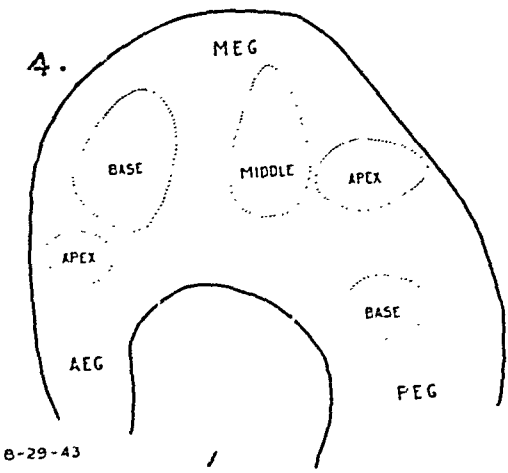
Fig. 2. Dorsal and ventral areas for frequencies designated at upper right. Right cortex; loudspeaker 0.75 meter from left ear; right cochlea destroyed. Only an occasional response from the sylvian gyrus. *AEG*, anterior ectosylvian gyrus; *MEG*, middle ectosylvian gyrus; *PEG*, posterior ectosylvian gyrus.

obtained anteriorly to 400, 800, 1600 and 2000; behind and dorsally to 800, 2000 and 4000. Frequencies of this ventral area were arranged in an inverse order to those of the dorsal area.

Ipsi-lateral side. The distribution of the responsive foci for the ipsi-lateral side resembles the contra-lateral side in detail.

Electrical stimulation (fig. 4). Stimulation of the ventral curve of the base of the cochlea with a condenser discharge at threshold strength gave a responsive area in the anterior end of the middle ectosylvian gyrus. With supra-maximal stimulus, an area was outlined upon the posterior ectosylvian gyrus. Stimulation of the middle coil produced a responsive area in the mid portion of the middle ectosylvian gyrus, with or without its area of high threshold on the anterior portion of the sylvian gyrus. The ventral area on the sylvian gyrus, when demon-





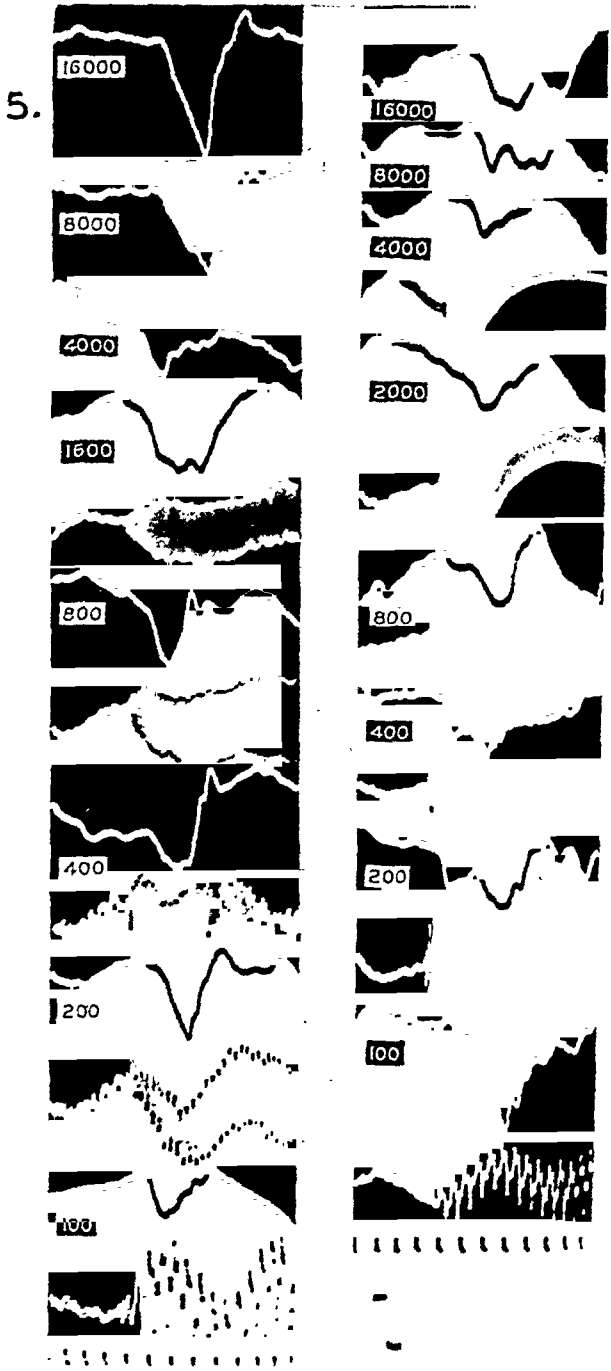


Fig. 3. Area of the dog cortex from which initially negative responses were most consistently obtained.

Fig. 4. Left cortex. Left cochlear spiral stimulated at the ventral points of the apical, middle, and basal turns. No responses from the sylvian gyrus.

For figures 3 and 4: *AEG* anterior ectosylvian gyrus. *MEG*, middle ectosylvian gyrus. *PEG*, posterior ectosylvian gyrus.

Fig. 5. Maximum responses obtained from the various areas illustrated in figure 2 to audio frequency stimulation. Left column: dorsal area. Right column: ventral area. The microphonic record of the sound is given below the cortical response except for 4000, 8000, 16000 of the dorsal area and 8000, 16000 of the ventral area, for which the carbon microphone was insensitive particularly at low intensities. Time: 60 cps. Amplitude: 400 microvolts (for responses only). Downward deflection: surface positive.

strated at identical stimulus values to those of the apex and base, occupied only a few square millimeters, with small responses. Stimulation of the apex activated a region on the posterior extremity of the middle ectosylvian gyrus and with stronger stimulation a focus upon the anterior ectosylvian gyrus. The dorsal and ventral areas were bilaterally represented with greater activation on the contra-lateral cortex for equal stimulus values.

Discussion. Ferrier (7) and Luciani and Tamburini (11) early concluded that the middle ectosylvian gyrus in the dog was an important landmark of the acoustic system, although Luciani (10) later found the cortical acoustic area to be more extensive. Munk (14) and Larionow (9) emphasized the temporal lobe as an "auditory sphere." Their physiological experiments, however, do not distinguish between the interruption of discrimination and that of association. The present investigation substantiates Luciani's (12) physiological demonstration for the existence of an "uncrossed" pathway to the cerebral cortex of the dog. Babkin (15) in Pavlov's laboratory found that absolute deafness supervened only when the anterior ectosylvian and middle ectosylvian gyri were included in their temporal lobe lesions. Pavlov attributed the loss to subcortical structures. Temporal lobe lesions involving the posterior portion of the area outlined in the present investigation disturbed transiently conditioned alimentary reflexes to individual tones, but seriously impaired discrimination of ascending and descending tonal scales. The experiment of Eliason, described in Pavlov's book, deserves mention. A reflex was established to a chord, 85-256-768 dv. Each component produced a reaction less than the entire chord but equal among themselves. The lesions, including the acoustic area of the present demonstration with the exception of the anterior portion of the anterior ectosylvian gyrus, resulted in loss of response to 768, but increased reaction to 85 and 256; 768 with re-enforcement reassumed its conditioning properties. The lesion removed the greater portion of the ventral area responsive to 800, leaving intact areas for 100, 200 and 400.

Results of audio frequency stimulation are supported by electrical stimulation of local regions of the cochlea. Both phases of the present investigation show in the dog that the extent of cortical localization and its divisions into two areas differing in location, threshold, and pattern are similar to the condition described in the cat by Woolsey and Walzl (21) by the electrical method. For their "primary" and "secondary" areas, dorsal and ventral, respectively, have been substituted to avoid implication of knowledge of the functions of such areas. Audio frequency stimulation adds information concerning localization which is unobtainable to electrical stimulation. As at the cochlear level (18), the arrangement of successive octaves at equal intervals is apparent in the dorsal area of the cerebral cortex. This was shown by Woolsey and Walzl (21) for the basal turn of the cochlea. The moderate concentration for 100 to 400 may be due to harmonic distortion since strong stimulus values were used. However, separation of foci indicates the activity of the fundamental. If the distance between two foci of excitation (or inhibition) in the cortex is necessary for the discrimination of two tonal stimuli, this suggests an explanation why the intervals of the

chord, thirds, fifths, eighths, etc. of the harmonic scale are recognized as equal or identical irrespective of pitch level.

The ventral area does not exhibit the same relation of interval to distance, but concentration of foci of the high and low frequencies with dispersal of the intermediate frequencies. However, within the areas of concentration, slight shifts of foci are evident. Such a pattern may be merely apparent because irradiation with intense stimuli, possible at several levels, namely, cochlear (8), geniculate (21) and cortical (1), could account for less discrete localization. Or, it may be real, for the development of the middle ectosylvian sulcus in the dog, together with the demonstration of responses within the sulcus, and the diminutive character of responses from the sylvian gyrus would indicate lengthening and dispersal of the cortical area for the intermediate frequencies, as well as reduction in the number of fibers to the sylvian gyrus. The responsive area in the present work agrees well with Campbell's (5) histological type for ectosylvian B cortex, but disagrees with the statement that the ectosylvian A cortex is the main projection area. His classification is supported by neuro-physiological investigations in other systems (2, 3, 13).

Retrograde studies (17) and Wallerian degeneration (20) on connections of the medial geniculate body with the cortex in the cat support the oscillographic studies of Woolsey and Walzl (21).

Consideration of the electrical sign of cortical afferents and latency measurements must be postponed until further data are available.

CONCLUSIONS

Foci responsive to audio frequency stimulation have been demonstrated in the cerebral cortex of the dog. These foci occupy the middle ectosylvian gyrus (dorsal area), high frequencies located anteriorly, low frequencies posteriorly. Successive octaves were arranged at equal intervals along the cortex.

A ventral area possessing an inverse arrangement of frequencies and a higher threshold was demonstrated with frequencies of 100 to 400 concentrated upon the anterior ectosylvian gyrus, 8000 to 16000 upon the posterior, and the intermediate frequencies dispersed over the sylvian gyrus and middle ectosylvian sulcus.

Each ear was represented bilaterally with the same pattern, but more strongly for the contra-lateral side.

Electrical stimulation of the bony spiral of the cochlea supports these observations.

Acknowledgment and gratitude are due Dr. Wm. F. Allen, whose devoted counsel and guidance have inspired this investigation. The author, also, wishes to acknowledge a special debt to Fred Claussen for his assistance in the problem.

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DEPRESSOR EFFECTS OF COLD UPON STATIC RECEPTORS OF THE LABYRINTH

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Received for publication March 2, 1944

With the usual type of caloric stimulation, the direction of the caloric nystagmus depends on the position of the horizontal semicircular canal (Bárány, 1911; de Kleijn and Lund, 1924; Dohlman, 1925). This is the strongest argument against Bartels' (1922) assumption that the caloric nystagmus is caused by a direct inhibitory action of low, and excitatory effect of high temperature upon labyrinthine receptors, and speaks in favor of Bárány's theory that the change in temperature produces an endolymph flow in the semicircular canals. Measurements of the temperature changes induced in the inner ear by douching the external auditory meatus (Meuman, 1924; Frenzel, 1925; Dohlman, 1925; Schmaltz, 1925), calculations (Schmaltz, 1925, 1932) and direct observations (Rossi, 1915; Maier and Lion, 1921; Steinhausen, 1931) support Bárány's theory. However, they do not exclude the possibility of other effects of the calorization upon the labyrinth, besides the production of an endolymph flow. Such by-effects are suggested by various experiences; e.g., caloric nystagmus could be elicited in pigeons in which the semicircular canals had been eliminated (Borries, 1921), or in some patients in whom rotation failed to produce ocular reactions (Mygind, 1925). Furthermore, Spiegel and Aronson (1933) observed on continuous caloric stimulation of the labyrinth that in some instances the position of the head failed to influence the direction of nystagmus. These observations could not be explained by Bárány's theory and were the starting point for the following experiments, in which the possibility of a depressor effect of cold upon labyrinthine receptors was tested, in cats and rabbits (experiments in 29 animals).

In a first series of experiments it was ascertained whether application of cold had such an effect upon the receptors in the ampullae of the semicircular canals.

Cats were rotated on a Bárány chair 10 times in 20 seconds and duration and frequency of the postrotatory nystagmus were determined before and during prolonged application of cold to both ears. For calorization, u-shaped cannulas were inserted in both external auditory meatuses, as symmetrically as possible; or they were pushed into the tympanic cavities after perforation of the drums. By means of a t-tube the cannulas were connected to a container that was filled with ice water and fixed about $1\frac{1}{2}$ m. above the animal, and by means of a second t-tube the outflowing water was collected. The temperature of the water was

[1] The work described in this paper was done under a contract, recommended by the Commitee on Medical Research, between the Office of Scientific Research and Development and Temple University.

measured before, and after, it passed through the cannulas. The mean of these two values permitted an estimate of the temperature of the cannulas.

These experiments failed to show significant differences in the duration and intensity of the postrotatory nystagmus before and after prolonged perfusion of the cannulas with cold water that was lowered from 20°C. to 3°C., even if the perfusion was continued for over an hour. In further experiments, the bilateral calorization was combined with application of a direct current of 5 to 10 milliamperes, both metal cannulas being connected to the anode, while a diffuse electrode was placed on the abdomen. However, also such a combination of cooling and "anelectrotonus" did not have a depressor effect upon the postrotatory nystagmus. The depressor effect of rotation upon the blood pressure (Spiegel and Démétriades, 1922) also remained practically unchanged following prolonged calorization.

In contradistinction to this negative result regarding the influence of cooling upon the labyrinthine reactions to rotatory stimuli, a definite effect of cold upon tonic labyrinthine impulses was observed in decerebrate cats. The animals were decerebrated under ether anesthesia by typical section through the midbrain, and then placed in supine position. A u-shaped cannula was placed in the external and middle ear on one side only and again was perfused with cold water. Water of 9° or below produced within a few minutes a decrease in the muscle tone of the homolateral foreleg, particularly in the shoulder, and to a slighter extent in the elbow muscles, causing a drooping of this leg; in some instances also the opposite foreleg showed a similar effect. This effect outlasted the calorization for from 5 to over 30 minutes, but was reversible. Similar, although less pronounced, effects could be observed in the extensor tone of the forelegs of rabbits with intact brain, the animals being held in recumbent position, with the head symmetrical to the trunk. Section of the trigeminal root or cocainization of the external meatus did not prevent these changes. They could also be observed when cannulas were placed into both external meatuses and tympanic cavities, and one side only was perfused with cold water, so that the effect of calorization could be compared with that of mechanical stimulation exerted by the cannula.

The effect of unilateral calorization upon the posture of the head was studied by keeping the u-shaped cannula, perfused with cold water, for several minutes in the external and middle ear and then quickly withdrawing it from the ear, so that the weight of the cannula and of its tubings filled with water did not influence the posture of the head. It was found that the cold temporarily produced effects similar to those seen in unilateral paralysis of the labyrinth: a rotation of the head about the oro-occipital axis with the affected ear lying below the normal one and turning of the head about a dorso-ventral axis toward the shoulder of the affected side. This could be demonstrated in unanesthetized rabbits (fig. 1) as well as in cats under bulbocapnine catalepsy.

The abnormal position of the head produced by unilateral labyrinthectomy in rabbits and cats was reduced by calorization of the opposite ear, but the head still remained somewhat inclined to the side of the extirpation. Occasionally

following unilateral application of cold in rabbits a phenomenon comparable to a "rebound reaction" was observed, the head being turned first to the affected, and then to the contralateral side, before it returned to the normal position.

In contradistinction to these depressor effects of cold upon static labyrinthine impulses influencing the tonus of the extremities and the posture of the head, the compensatory deviations of the eyeballs were not influenced by the calorization. These latter reactions were studied in rabbits. Head and body of the animals were fixed on a board, a linear scar was produced in the cocainized cornea by cautery, and the board with the animal was slowly tilted around a bitemporal axis of the head from the horizontal into an oblique position by lifting the caudal end of the board. Usually an angle of 38° to 40° was attained between the board and the horizontal plane. The angle between the corneal linear scar and a vertical line was measured by a spy glass in the various positions of the animal. No significant differences between the compensatory deviation before and during prolonged bilateral calorization could be observed. The counter-rolling of the eyeballs was 15 to 26° before, 14 to 27° after prolonged calorization. This

FIG. 1. Rabbit. Calorization of right ear (2° C.) (a, left) before calorization; (b, right) immediately after calorization (2 min.).

negative result is in agreement with experiments by deKleijn and Storm van Leeuwen (1917) on rabbits subjected to cold water irrigation of short duration.

The threshold of the vestibular nerve for galvanic stimulation was tested by monaural or binaural stimulation before and during prolonged calorization. The application of cold failed to change this threshold (e.g., nystagmus toward the cathode at 1 milliampere with as well as without bilateral perfusion of the cannulas with water of 3°C.).

DISCUSSION. These last experiments indicate that the effects of cold described in this paper can not be caused by a change of the excitability of the vestibular nerve, since the vestibular reaction to galvanic stimulation depends on the state of the vestibular nerve; it is lost after intracranial section of this nerve (Dohlman, 1929; Spiegel, 1942), but practically unchanged after labyrinthectomy (Neumann, 1910). Thus the depressor effects of cold must be ascribed to an effect upon the peripheral labyrinthine receptors. It may seem surprising that the application of cold affected only a part of the labyrinthine functions. It decreased some static impulses that are supposed to originate in

the utricular macula, while the excitability of the receptors for angular acceleration, the cristae ampullares, and that of the receptors for the compensatory eye deviations[2] was not changed.

This experience, however, is not without analogy. It should be remembered that Arndts (1926), McNally (1927) observed an isolated decrease of muscle tone in the homolateral limbs without impairment of other labyrinthine reflex functions following puncture of the round window in rabbits. The effects of puncture of the round window are of interest also for the further analysis of the effects of cold. The possibility should be borne in mind that these effects could be caused only partly by a direct depressor action of the cold upon labyrinthine receptors, and partly by indirect effects of the decrease in temperature. One possibility is a decrease in intralabyrinthine pressure, which may at least in part be caused by vasoconstriction. It was, therefore, tried to ascertain the effect of perfusion of the external meatus with cold water upon the intralabyrinthine pressure, using Hughson's (1932) method of estimating this pressure in cats.[3]

These experiments, however, did not give a completely satisfactory result. Although in some cases a slight fall of pressure was observed on prolonged calorization, the pressure did not regularly return to the initial value after the application of cold was stopped, apparently because in these prolonged experiments a slight leakage between the needle of the manometer and between the bone could not be completely avoided. It was, therefore, tried to approach the problem in a different way, viz., by comparing the effect of unilateral application of cold with that of contralateral decrease of intralabyrinthine pressure by puncture of the round window. Such a comparison showed in rabbits that the effect of calorization was more extensive than that of the puncture. While the latter operation produced, in agreement with the findings of Arndts, McNally, only a decrease of tonus in the ipsilateral limbs, particularly in the foreleg, the application of cold induced also a change in the posture of the head that became inclined to the cooled side.

Thus the depressor effect of cold upon static labyrinthine impulses can not be explained by decrease of intralabyrinthine pressure alone, although a possible participation of this factor should not be denied. Furthermore important objections against Wittmaak's hydrostatic theory of direct stimulation of the vestibular receptor cells have been raised by Steinhausen (1935). This author points out that a direct excitation could only be produced by a hydrostatic pressure above 300 atmospheres. He tries to approach an understanding of the function of the otoliths by calculating the pressure exerted by them upon the macula. This pressure, A, equals the weight of the otolith in air, W, minus the weight of the displaced endolymph $\frac{W}{S}$. (The specific gravity of the latter s is

[2] The receptor apparatus for these reflexes is uncertain; Lorente de Nó (1931) points out that not only the otoliths, but also the semicircular canals may participate in the genesis of these reactions.

[3] In some of these experiments the water bubble manometer devised by Dr. Rubin Lewis was used. I wish to express my appreciation to Doctor Lewis for lending me this manometer and advising me as to its use.

assumed to be equal to that of water.) The specific gravity of the otolith, S, is according to Ulrich's (1935) measurements on the pike 2.93. In view of the fact that the otoliths have the same chemical constitution ($CaCO_3$, aragonite crystals) in various species, this value may be used in the following equation:

$$A = W - \frac{W}{S} = W\left(1 - \frac{1}{2.93}\right) \qquad (1)$$

At the temperatures T_1 and T_2 differing by t^0, $A_1 = W\left(1 - \frac{s_1}{2.93}\right)$;

$$A_2 = W\left(1 - \frac{s_2(1 - ct)}{2.93}\right)$$

$$A_1 - A_2 = W\left(\frac{s_2(1 - ct) - s_1}{2.93}\right) \qquad (2)$$

In these equations, s_1 and s_2 are the specific gravity of water (endolymph) at the temperatures T_1 and T_2. c is the coefficient of cubical expansion; it is for marble $0.3\text{–}0.6 \times 10^{-4}$.

The measurements of Schmaltz (1932) show that syringing of the external ear with 1000 cc. H_2O of about 10° produces a drop of temperature in the inner ear of about 4°. Thus $t = 4°$; s_1 at 38° equals 0.99299 and s_2 at 34° 0.99440. Substituting these values in equation (2) one arrives at a value $A_1 - A_2 = 4.1 \times 10^{-4}W$.

The question arises whether such a change of otolithic pressure upon the hairs of the macula reaches the threshold. Unfortunately few data are available that would permit one to estimate this threshold. Grahe (1927, 1932) found that deviations of 1–3° in the position of the head from the vertical (in the frontal plane) can be perceived. In these experiments the head was in fixed relationship to the trunk so that asymmetrical impulses from the neck muscles were excluded. Although the part played by proprioceptive impulses from other parts of the body in the perception of position should not be overlooked, the importance of the labyrinth is indicated by the experience that following unilateral elimination of this organ the perception of the vertical direction is shifted toward the affected side. The significance of the otoliths in the perception of position was strikingly demonstrated by studies of Quix and Eijsvogel (1929). These authors found that subjects are able to estimate with great accuracy the inclination to the vertical direction in those positions in which the otoliths press upon the maculae, but make grave errors in positions in which otolithic pressure upon the maculae is absent. It may, therefore, be of interest to calculate the change in otolithic pressure, if the head is brought from the normal into an oblique position.

If we assume that the otolith of the utricle is approximately horizontal in the normal position of the head, so that the pressure A_v of the otolith acts in the vertical direction, inclination of the head by α degrees will be associated with an inclination of the macula of the same extent. The pressure of the otolith in the direction perpendicular to the macula will be $A_o = A_v \times \cos \alpha$, and the dif-

ference of pressure associated with a perception will be $A_v - A_o = A_v (1 - \cos \alpha)$. According to equation 1, A_v equals $0.66W$. For an angle of 2°, $A_v - A_o = 0.66W \times 6 \times 10^{-4} = 3.96 \times 10^{-4}W$. For an angle of 3°, $A_v - A_o = 0.66W \times 14 \times 10^{-4} = 9.24 \times 10^{-4}W$. Thus the change in otolithic pressure produced by the cooling of the endolymph, $4.1 \times 10^{-4}W$, seems to lie within the range of or close to the pressure changes accompanying the inclination of head and body in Grahe's experiments. The cooling effect in the present experiments probably was more intense than in the instances measured by Schmaltz, since he douched the external meatus, while here the cannula usually was pushed into the tympanic cavity, and the perfusion was often continued for a much longer time. It seems, therefore, not impossible that, besides a direct effect of the cold upon labyrinthine receptors, simple physical effects of the cooling could at least partly be responsible for the depressor effect of cold upon tonic labyrinthine impulses.

SUMMARY

A depressor effect of cold upon tonic labyrinthine reactions is demonstrated in cats and rabbits (decrease of the extensor tonus on the side of the cooling, inclination of the head toward this side). The possible rôle of physical factors in this phenomenon is discussed.

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RECOVERY OF THE CEREBRAL CORTEX OF THE CAT FOLLOWING HYPOXIA

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Received for publication March 4, 1944

Although temporary interruption of the blood supply to the brain may cause persistent signs of cerebral cortical damage (Kabat, Dennis and Baker, 1941, among many other investigators), it is not clear whether exposure to an atmosphere low in oxygen tension, in the absence of complicating factors such as depression by anesthesia (Courville, 1941), can produce permanent impairment in the surviving mammal (cf. Armstrong and Heim, 1938; Ford, 1928; Himwich et al., 1938; Horvath et al., 1941, Levine and Schilder, 1940; Thompson and Corwin, 1938; Armstrong, 1939). The studies reported here were undertaken to define the extremes of duration, intensity, and repetition of hypoxic exposures to which cats could be subjected without permanent central nervous impairment.[2] Many of the tests were performed in connection with studies of the effect of thiamin deficiency and reduced food intake on resistance to hypoxia (Smith, Oster and Toman, 1944).

METHODS. Unanesthetized mature cats in good health were placed in an observation chamber through which nitrogen-air mixtures were passed at a rate of flow which reduced chamber oxygen tensions to within less than 1 per cent of final value within five minutes. Except where otherwise noted, animals were removed to air and revived by artificial manual respiration when breathing had failed to the point of occasional gasps or had stopped completely. The length of time that respiration could be maintained was taken as the measure of the survival time of the animal. This endpoint was suitably sharp and was heralded one to two minutes earlier by a rapid decline in respiratory rate from the hyperpneic level. Resuscitation was usually successful unless withheld for more than two minutes. In surviving animals, recovery time of reflexes, postural reactions, and behavior of individuals were followed. In some animals suffering permanent impairment, electrical activity of the cerebral cortex was recorded with an ink-writing oscillograph, using scalp electrodes in the unanesthetized animals or saline wick electrodes applied to the pia under barbiturate anesthesia; brains were fixed by intra-arterial perfusion with formol-acetic-alcohol in animals freshly sacrificed, and paraffin sections were stained with toluidine blue for examination of cells.

RESULTS. *Induction.* When placed in the chamber the cat would usually remain quiet for the first two to three minutes. During the next two to three minutes the animal exhibited signs of restlessness (such as mewing, turning

[1] Aided in part by a grant from the Bressler Alumni Research Fund.

[2] Preliminary report, Federation Proceedings 2: 37, 1943.

around), profuse salivation and defecation almost invariably occurring. Less frequently, urination was seen. Inability to maintain an erect posture occurred at four to six minutes after the beginning of the exposure and shortly thereafter unconsciousness supervened. When the animal survived 10 minutes or longer, it occasionally revived long enough to lift its head, mew, or even struggle. In the early part of the test a "startle" response to a sharp tap on the side of the box was greatly augmented. Later this response declined to nothing more than a slight pupillary dilatation.

Survival. When resuscitation was begun at the point of respiratory failure, only 6 deaths resulted from 300 hypoxia tests on 70 animals. The low mortality made possible repeated trials on individual animals. In the 6 fatal cases, a palpable heart beat was feeble or absent during attempted resuscitation. Table 1 summarizes the survival data for 56 cats on their first exposure to the indicated oxygen concentrations. Data for the intermediate range are represented graphically in figure 1. The estimated corrections are for time consumed in reaching

TABLE 1

Survival times of normal cats exposed to low oxygen concentrations

O_2 (%)	0	2.30–2.50	3.00–3.19	3.20–3.39	3.40–3.59	3.60–3.79	3.80–3.99	4.00–4.19	4.50–5.00
No. of Cats	[illegible]	5	8	6	13	8	5	4	2
Range of survival times (min.)	4–6	5–12	16–46	18–53	14–60	14–64	15–87	34–60	Above 120
Mean survival time	5.2	7.4	25.1	27.7	29.8	31.7	36.2	45.2	
Standard error of mean	0.4	1.3	3.3	4.9	3.7	5.6	13.8	5.8	
Estimated correction of mean	−3	−4	−5	−5	−5	−5	−5	−5	
Corrected mean	2.2	3.4	20.1	22.7	24.8	26.7	31.2	40.2	

the lethal range during the exponential washout period. Standard errors of the uncorrected means were approximated by a method for small samples without correction for skewness. Only initial tests were used in constructing the survival data. since acclimatization (increase in survival time) of as much as 80 per cent had been observed in some animals at the end of a week of daily trials. No such acclimatization was seen when tests were spaced a week apart.

Recovery. Early reflex recovery was similar except in time-scale to that described by Kabat et al. (1941) in the dog following interruption of cerebral circulation. In general, recovery proceeded from the head caudad, extensor responses preceded flexor in the same limb, and lower centers recovered before higher. If animals had been promptly resuscitated, vision and the hopping and placing reactions returned within 10 minutes. In the ensuing hour, such normal activities as cleaning of fur were undertaken and residual ataxia disappeared. However, alterations in behavior and general activity of individual animals remained for about two hours. In some cats this was characterized merely by

sluggishness. In many there was a period of abnormally enhanced affectionate behavior toward persons in the room. A few normally gentle animals regularly fought on return to the group. One animal consistently suffered a prolonged epileptiform seizure.

Times of recovery of reflexes and postural reactions were followed in one animal at six oxygen concentrations from 0 to 4.2 per cent giving survival times of 4 to 87 minutes. There was little variation within this range. For instance, the foreleg contact placing reaction returned at approximately 8 minutes throughout. In other animals the similar time course of recovery suggested that it was to a large extent independent of intensity and duration of exposure within the range studied, provided a uniform end-point of respiratory failure was used.

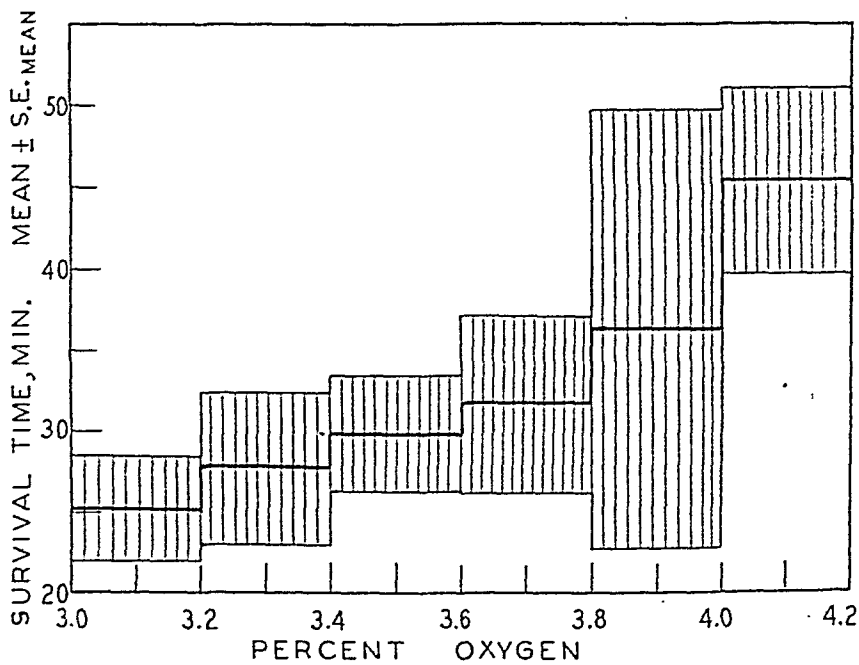

FIG. 1. Duration of exposure necessary to abolish respiration in normal unanesthetized cats. Ordinate: heavy line, mean survival; shading, standard error of mean. Abscissa: ranges of chamber oxygen samples. Data from initial tests on 49 animals.

Recovery was complete and rapid in all those animals exposed for the first time or repeatedly exposed at intervals of a week, if they were resuscitated promptly at the point of respiratory failure. Recovery was complete but delayed in animals whose cardiac recovery was delayed up to 90 seconds following respiratory failure. Recovery was complete in most animals after repeated daily exposures; for example, one cat subjected to 3.2 per cent oxygen 6 days a week for a total of 23 trials recovered with usual rapidity from the last trial. One animal recovered completely and rapidly from 15 consecutive trials in one day with pure nitrogen, recovery between trials being permitted only to the point of attempted head-righting.

Permanent central nervous damage. Five animals after from 12 to 21 daily hypoxia survival trials (usually 3.5 per cent oxygen) showed lasting impairment characterized by blindness, loss of hopping and placing reactions, and general depression of activity. These were animals in which cardiac recovery was slow

following the last exposure. Three became moribund within a week. The other two were maintained for several months and recovered from the major disabilities, but developed a permanent hyperactivity and a stereotyped pattern of cage-scratching, which was repeated continually by the most affected animal except when feeding.

Brain sections of the two long-surviving animals showed scattered cell damage from cerebral cortex to medulla. The small percentage of cellular pathology observed was probably related in part to the long period intervening between the last trial and sacrifice of the animal. In the cerebral cortex, upper layers of the visual cortex showed most destruction; large pyramids of the motor cortex were spared while upper layers showed definite involvement. Of subcortical centers, the lateral geniculates were most affected.

TABLE 2

Cardiac and cerebral cortical recovery following respiratory failure and delayed artificial respiration

CAT NO.	% O_2	TIME AFTER RESPIRATORY FAILURE		
		Began artificial respiration	Cardiac recovery	Recovery of foreleg contact placing reaction
		sec.	*sec.*	*min.*
1	0	90		
2	2.5	90	520	
3	0	145	185	
4	0	120	175	*
5	0	120	150	45
6	2.5	55	145	
7	2.5	60	135	
8	2.4	30	90	18
9	0	50	85	19
10	2.5	45	80	15
Control	2.5	0	0	7.5†

Blank space = died before recovery.
* Functional decorticate, survived a week.
† Mean of 8 determinations, range 6 to 10 minutes.

In three animals showing persistent signs of cortical damage, electrocorticograms revealed continuous slow dysrhythmia with 3 per second waves predominating, in contrast to the usual bursts of 6 to 10 per second rhythm with intervening quiet periods in control animals at the same level of barbiturate anesthesia. Electroencephalograms of the conscious animals were inconclusive because of individual variations in controls.

A series of 10 previously unexposed cats were tested in the usual manner except that artificial respiration was purposely withheld for periods from 30 to 145 seconds. A palpable heart beat returned in all but one animal, but failed again after a few minutes in 4. Of 5 survivors, 4 had a complete but delayed central nervous recovery, while a single animal remained in the functionally decorticate state for eight days, then died. The results are summarized in table 2, where animals are arranged in descending order of delay in cardiac recovery.

DISCUSSION. Weinberger et al. (1940) reported irreversible damage to the cerebral cortex of cats after a six-minute interruption of circulation. The present studies indicate complete recovery from hypoxia over the entire range of values just adequate to abolish respiration, provided an additional period of complete anoxia due to circulatory failure is avoided. Tissue oxygen tensions must have been quite low even in the upper part of this range; Courtice (1941) found cerebral venous oxygen tension to be about 9 mm. Hg pO_2 in chloralose-anesthetized cats subjected to 6 per cent oxygen in inspired air; in our animals 6 per cent oxygen failed to suppress the "startle" response to sound in several hours, although impairing postural reactions and inducing seizures.

The difficulty experienced in producing permanent central nervous damage by critical exposures to low oxygen tension in inspired air suggests that the margin of safety between the time of appearance of such damage and of irreversible circulatory failure is a narrow one under these conditions, as Ford (1928) had concluded from similar studies. Possibly the margin may be widened somewhat by cumulative effects of previous exposures, but cases of permanent damage in the present report were associated with delayed cardiac recovery following the final exposure.

SUMMARY

1. Survival data are given for cats breathing nitrogen-air mixtures containing up to 4.5 per cent oxygen. Higher concentrations permitted respiration for two hours or more.

2. Within the lethal range, exposures for a period just sufficient to abolish respiration did not produce signs of permanent functional cortical damage provided manual artificial respiration was promptly given and cardiac recovery was not delayed.

3. A few cases of permanent central nervous damage were produced after repeated daily lethal exposures or delay in resuscitation following a single exposure. These were associated with delayed cardiac recovery and a consequent period of complete anoxia added to the duration of the preceding hypoxia. Electrocorticograms from these damaged animals show abnormal waves of the three per second type.

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THE EFFECT OF SODIUM THIOCYANATE ON THE PRESSOR ACTION OF A RENIN-LIKE SUBSTANCE

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Received for publication February 15, 1944

The inactivation of pressor substances by oxidation has been described. Renin was destroyed by heat (1) and activated renin or angiotonin was destroyed by strong oxidizing agents (2). Pressor amines have been inactivated by enzyme amine oxidase (3) and amine oxidase injected into rats eliminated renin response (4). Adrenalin, a pressor amine, has been inactivated by ortho substituted phenols in a tyrosinase system (5). Although potassium thiocyanate has been shown to cause a slight reduction in hypertension experimentally produced in dogs (6), the dose was proportionately 50 times that in man. Renin has not been detected in the blood of normal dogs intoxicated by potassium cyanide (7). Dopa quinone obtained from dihydroxyphenyl-alanine by oxidation with potassium ferricyanide at pH 8 destroys pressor amines more effectively than does aeration (8).

PURPOSE. It is the purpose of this paper to demonstrate the effect of sodium thiocyanate on the pressor response of single doses of a renin-like substance (hereafter called renin) injected intraperitoneally into rats.

METHODS AND RESULTS. A crude extract of kidney substance was used which is soluble in water at a pH 4.0 and in 50 per cent acetone and insoluble in ammonium sulphate solution and in 85 per cent acetone solution. Five milligrams of this amorphous substance were equivalent to 1 gram of fresh kidney substance. Injected intraperitoneally into each of six rats, 5 mgm. of this renin caused a rise in blood pressure over 30 mm. above the average pressure within an hour. This was the prerequisite set forth by Freedman (9). A modified Grollman pressure apparatus was used.

These rats were then given subcutaneous injections of 20 mgm. of sodium thiocyanate per 100 grams body weight each day for ten days. One hour after the last injection, the intraperitoneal injection of 5 mgm. of renin was repeated and the pressure readings were taken every ten minutes for ninety minutes. The sodium thiocyanate was discontinued for one week and the intraperitoneal injection of 5 mgm. of renin was repeated and the pressure readings were recorded in the same fashion.

The average blood pressure for each rat had previously been established on a basis of at least ten readings taken over a period of a month. This average was subtracted from the average of one rise in pressure elicited by the renin injection. All such differences at each ten-minute time interval were averaged. By applying the t- formula of Fisher (10), the significance of differences between the

[1] The authors are indebted to Dr. Fritz Bischoff for valuable suggestions and to Miss Georgena Clarke for preparation of a renin-like kidney extract.

means of the respective groups treated with renin alone and those in which renin followed the subcutaneous injection of sodium thiocyanate was established. All factors for P which are less than 0.05 indicate differences that are significant. For a graphic expression of these results see figure 1.

A week following discontinuation of the sodium thiocyanate 5 mgm. of renin were injected again intraperitoneally and in each instance adequate pressor effect was recorded. Although local reactions at the site of injection were in no in-

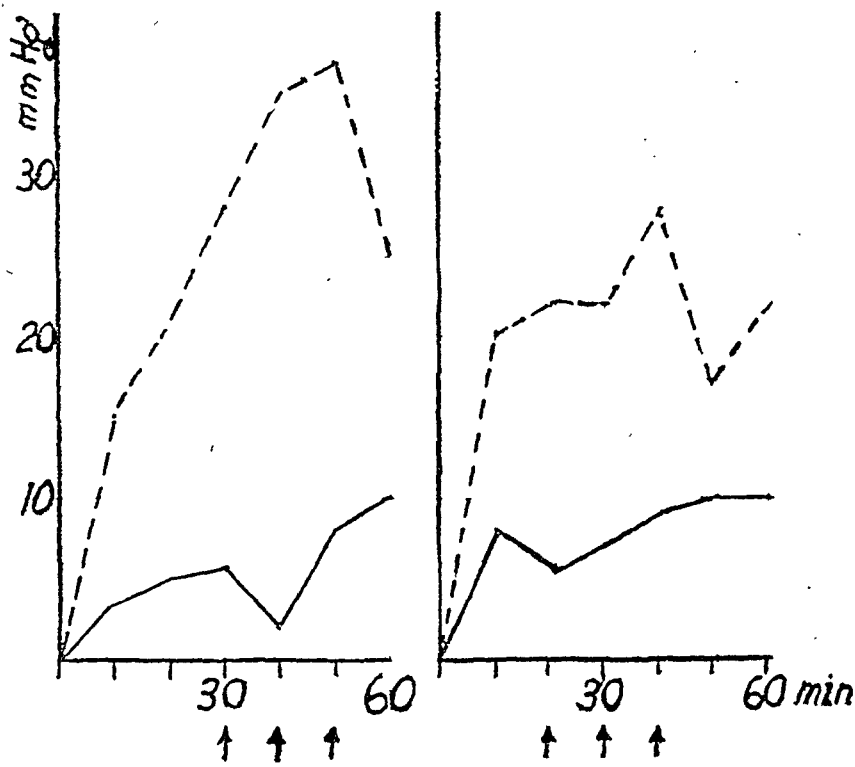

FIG. 1 FIG. 2

Figs. 1 and 2. Broken line indicates average blood pressure rise above the normal mean at a given 10-minute interval after the injection of renin.

Solid line indicates average blood pressure rise above the normal mean at a given 10-minute interval after the injection of renin following the injection of sodium thiocyanate in figure 1, and following sodium thiocyanate by mouth in figure 2.

Arrows indicate points of significant difference between these means. The P factors in figure 1 are respectively <0.05, <0.01 and <0.05. The P factors in figure 2 are respectively <0.05, <0.02, and <0.05.

stance remarkable, sodium thiocyanate was given by mouth in drinking water to obviate the criticism that the anti-pressor effect was due to non-specific tissue reaction as described by Friedman (6).

A minimum of 20 mgm. of sodium thiocyanate per 100 grams body weight was ingested each day in drinking water for ten consecutive days. On the morning of the eleventh day, 5 mgm. of renin were injected intraperitoneally and the pressure readings were recorded at ten-minute intervals as in the first experiment. The differences between these readings and the established mean were compared with averages at the same time interval when renin was injected alone. The same statistical treatment of data to determine the significance of differences was made with the results as portrayed in figure 2.

Discussion. Following the method of Freedman (9) to determine the pressor effect of renin-like material, it was found in each instance that the rise in blood pressure was equal or greater than 30 mm. as was described as an essential criterion. These points of maximum reaction occurred at varying intervals of time after the injection of renin. Thus the average at any given ten-minute interval was a little less than the 30 mm. prescribed. However, the existence of definite difference in means in the central portions of both curves seems conclusive evidence of the reducing effect of sodium thiocyanate on renin pressor reaction. Since the discontinuation of the sodium thiocyanate allowed the renin pressor response to occur again, it would seem that a mechanism such as desensitization was ruled out as a cause of the reduction in pressor effect of renin.

Sodium thiocyanate blood level determinations were run on six rats after a series of injections of thiocyanate similar to those in the first experiment. One hour after the last ten consecutive daily doses of sodium thiocyanate were given, blood level determination was made on blood obtained by intraventricular puncture under ether anesthesia. In each instance, the blood level was 20 mgm. per cent. Although this level was present in all rats at the end of ten days, two rats required additional ten and fifteen days' dosing before renin pressor effect was diminished. Twenty-four hours after discontinuation, the blood levels of sodium thiocyanate was 0 mgm. per cent. In four of these rats the reduction of renin pressor effect continued for from one to seven days. In each of these four rats the pressor effect was present by the eighth day following the discontinuation of thiocyanate.

It was found that the blood levels of thiocyanate in rats receiving the drug by mouth ran between 6 and 12 mgm. per cent. These rats at these levels showed uniformly an obliteration of the pressor effect.

CONCLUSION AND SUMMARY

1. By subcutaneous injection or by mouth, sodium thiocyanate will minimize the pressor effect of unit amounts of a renin-like substance when injected intraperitoneally into normal and sensitized rats.

2. There is some indication that sodium thiocyanate does not act directly to perform this function but that it may activate some other mechanism in the body which is not so easily dissipated as sodium thiocyanate and which will continue to eliminate the pressor effect.

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THE EFFECT OF CHROMATOLYSIS ON OXYGEN CONSUMPTION IN THE SPINAL CORD OF THE GUINEA PIG

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Received for publication January 17, 1944

Since its first description by Flemming in 1882 the Nissl substance has figured prominently in cytological investigations of nerve cells. The striking changes in the material brought about by the so-called axon reaction or by other methods have received careful and detailed study. Yet the rôle of this conspicuous cell component in normal and altered metabolic states continues to remain obscure. Within recent years pertinent data have been contributed by Bensley and Gersh (1933) and by Caspersson (1940, 1941) on the chemistry of Nissl substance, by Gersh and Bodian (1943) on the chemical changes associated with chromatolysis, and by Turner (1943) on the actual numbers of cells involved in the axon reaction. In the light of these newer investigations it became apparent that further useful data might be obtained by comparing the oxygen consumption of normal spinal cord with that in which the anterior motor horn cells had been altered by axon reaction.

MATERIALS AND METHODS. In one series of experiments transverse slices of spinal cord, taken between L5 and S3 segments, were removed from twenty-one male guinea pigs of approximately equal weight. Grouping was such that 50 samples were from normal animals (group 1), 36 from animals in which bilateral chromatolysis had been induced by section of both sciatic nerves fifteen days previously (group 2), and 30 from animals whose sciatic nerves had been bilaterally sectioned three months previously (group 3). A second series of determinations utilized 10 male animals in which the sciatic nerve of only one side had been sectioned fifteen days previously. In this second series the segments of spinal cord from L5 to S3 were first divided sagitally and 30 samples from the side of the cord on which the nerve had been sectioned were run against comparable samples from the unoperated side as controls. A third series of experiments was carried out in which the oxygen consumption in segments L5 to S3 was measured on the 1st, 2nd, 3rd, etc., days following bilateral section of the sciatic nerves. Slicing was done inside a large box maintained at 8 to 10°C. and saturated with water vapor. All tissue slices were placed in Dickens-Greville Ringer solution buffered to pH 7.4, and the oxygen consumption over a period of 80 minutes was measured in a Warburg apparatus at 37.5° C. (with precautions suggested by Dixon, 1943). The samples of tissue were placed in the vessels in such a way that the oxygen consumption of comparable cord levels was measured in comparable vessels in each case.

[1] Aided by a grant from the Kinney Fund and from the Fluid Research Fund, Stanford University School of Medicine.

RESULTS. The mean rates of oxygen consumption (expressed as milliliters of oxygen at normal pressure and temperature per gram wet weight of tissue per hour) of all samples from the three groups in the first series are shown graphically in figure 1. The oxygen consumption of the group with bilateral chromatolysis (group 2) and of the bilaterally operated chronic group (group 3) is significantly below the normal (group 1). The calculated P value (Fisher, 1936) in this case is less than 0.00001—i.e., such a difference would be expected by chance alone only once in a hundred thousand times. The difference between group 2 and group 3 is negligible (P greater than 0.9). As has been noted by other workers, the increasing ratio of grey to white matter from L5 caudalward is reflected in a progressive increase in oxygen consumption. This is demonstrated in figure 2 in

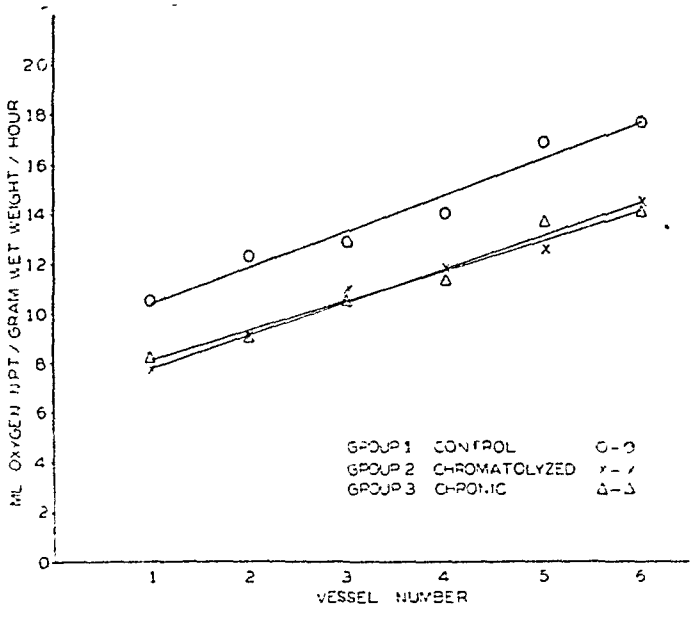

Fig. 1. Mean rates of oxygen consumption of segments L5-S3 of the spinal cords of guinea pigs in the three groups of the first series, plotted for each manometer vessel used. Vessel 1 contained tissue from the region of L5, vessel 2 contained more caudal tissue, and so on to vessel 6 which contained tissue from the region of S3.

which vessel 1 contained tissue from segment L5, vessel 2 contained more caudal tissue, and so on to vessel 6 which contained the tissue from the region of S3. The fact that the position of these curves for oxygen consumption in the various vessels did not change significantly with respect to one another within the three groups in the first series might be taken as presumptive evidence that the cell changes in groups 2 and 3 were fairly evenly distributed among the segments of the spinal cord studied.

Data from the second series of experiments are summarized in table 1, in which the mean oxygen consumption of the half segments of the spinal cord from the operated side is contrasted with that of the half segments from the unoperated side. The difference is slight (0.036) and statistically insignificant (P greater than 0.4). It should be noted that the values for mean oxygen consumption in

this series fall within the range of values for groups 2 and 3 of the first series—i.e., are significantly below the normal (group 1) mean.

The rates of oxygen consumption in these series are of the same general order of magnitude as reported for the spinal cord of the cat by other workers (van Harreveld and Tyler, 1942; Craig and Beecher, 1943).

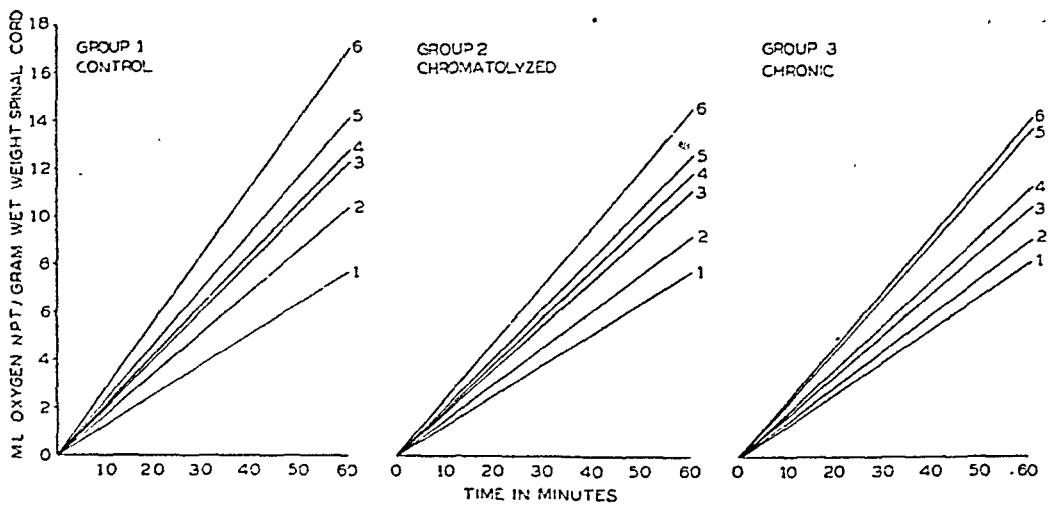

Fig. 2. Mean rates of oxygen utilization in the three groups of the first series, plotted for each manometer vessel used. The tissues were placed in the vessels in the same arrangement as indicated for figure 1.

TABLE 1

NO. OF SAMPLES	MEAN QO_2		
	Operated side	Unoperated side	Difference (U − O)
30	1.0788	1.1145	0.0357

Discussion. Inspection of the data from the first series of experiments would indicate a significant depression in the oxygen consumption in segments L5 to S3 of the spinal cord in those animals with bilateral chromatolysis (group 2) and in those animals with chronic bilateral sciatic nerve section (group 3). Although it has been shown (Turner, 1943) that some 38 per cent of the anterior motor horn cells (e.g., in segment L5) are undergoing chromatolysis fifteen days after sciatic nerve section, the depression of QO_2 exhibited in group 2 cannot be accounted for solely on this basis since no such difference was observable between the operated (chromatolysed) and unoperated (control) sides of the spinal cords in the second series. The depression of oxygen consumption in the bilaterally operated chronic group (group 3) is also difficult of explanation. It is known (Turner, 1943) that there are approximately 16 per cent fewer anterior motor horn cells in segment L5 of such chronically operated animals (and presumably a comparable decrease in other segments contributing to the sciatic nerve), yet the fact that between groups

2 and 3 there is no significant difference in oxygen consumption and the fact that both operated and unoperated sides in the second series showed a lowered QO_2 would again suggest that decrease in number of cells alone could hardly account for such depression. These observations present a suggestive parallel to recent investigations by Nachmansohn and Hoff (1944) who have shown that either unilateral or bilateral deafferentation brings about a decrease of 10 to 20 per cent and 30 per cent, respectively, in choline esterase activity in all four quadrants of segment L6 of the spinal cord of the cat. With regard to the present results, the possibility exists that depression in the energy made available by oxidation is compensated by increased energy supplied by anaerobic glycolysis (Pasteur effect, cf. Burk, 1939). Data on anaerobic glycolysis under conditions comparable to these experiments are not yet available.

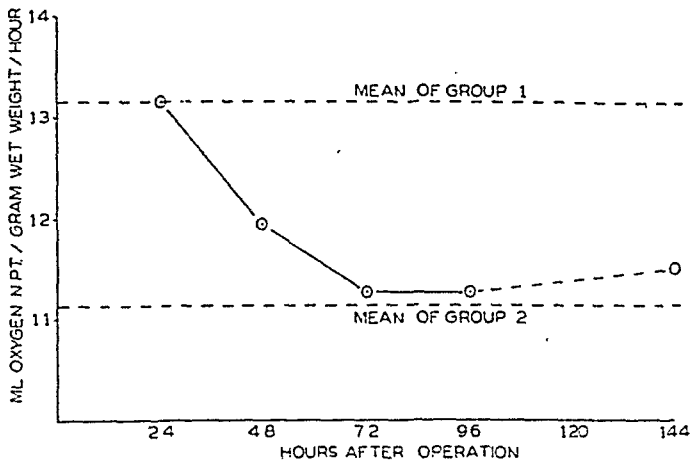

Fig. 3. Mean rates of oxygen consumption of segments L5-S3 of the spinal cord plotted against time in hours after section of both sciatic nerves. Dotted lines indicate the mean oxygen consumption of the control group (1) and the group measured 15 days after bilateral section of the sciatic nerves (2).

The time of appearance of the depression of oxygen consumption following bilateral sciatic nerve section is of some interest. As will be seen in figure 3, by the third day after operation the oxygen consumption has fallen to the level of that seen on the fifteenth day. It is perhaps suggestive that Gersh and Bodian (1943), utilizing the techniques of Caspersson (1940, 1941), have shown that the rate of disappearance of certain nucleotides and protein constituents from nerve cells undergoing chromatolysis follows a somewhat similar time course. Gersh and Bodian postulate an increased activity of ribonuclease (or faulty synthesis of nucleotides) to account for changes in the Nissl substance observed during chromatolysis. Either of these processes might well reflect in decreased action of various enzyme systems of the cells (specifically, perhaps, co-enzyme 1). Hence a depression of QO_2 during chromatolysis cannot with certainty be attributed to a simultaneous decrease in Nissl substance alone. It is well recognized that the disappearance of Nissl substance is but a visible reflection of one of many possible aspects of the cell state during chromatolysis.

SUMMARY

1. Segments L5 to S3 of the spinal cord of guinea pigs in which bilateral chromatolysis of anterior motor horn cells had been induced by section of both sciatic nerves fifteen days previously showed a depression of oxygen consumption significantly below normal.

2. Segments L5 to S3 of the spinal cord of guinea pigs in which the sciatic nerves had been bilaterally sectioned three months previously showed a similar significant depression in oxygen consumption.

3. Fifteen days after chromatolysis had been induced on one side of the spinal cord by unilateral section of the sciatic nerve, comparison of the opposite halves of segments L5 to S3 showed a difference in oxygen consumption which was not statistically significant.

4. From these data it would appear that neither the estimated number of chromatolysed anterior motor horn cells of segments L5 to S3 nor the consequent decrease in actual numbers of these cells can adequately account for the lowered oxygen consumption observed fifteen days and ninety days, respectively, after section of the sciatic nerves.

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THE EFFECT OF SODIUM AND POTASSIUM CHLORIDE ON THE RENAL CLEARANCE OF ASCORBIC ACID

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Received for publication December 31, 1943

It has been found that estradiol benzoate increases the renal excretion of ascorbic acid by depressing the tubular reabsorption of this substance at load levels which normally effect tubular saturation (1). Since similar disturbances in the renal excretion of certain electrolytes (sodium and potassium chloride) follow the administration of estradiol (2), possibly as the result of alteration of the renal tubular mechanism for reabsorption of these substances, the mechanisms of tubular transfer or ascorbic acid and these salts may be related in some way. In the present report, this point has been investigated by simultaneously infusing ascorbic acid and either sodium or potassium chloride. Utilization of a common transfer mechanism should then result in interference with tubular reabsorption of one of the substances as the other occupies the transfer mechanism, as has been demonstrated by Shannon for the relationship of glucose and xylose (3), and more recently by Pitts (4) for the relationship of glycin and creatine.

Ascorbic acid shows the characteristic of being filtered at the glomeruli and actively reabsorbed by the tubules, the latter being limited by a maximal rate (Tm) in dog and man (5, 6). The magnitude of the ascorbic acid Tm is small in dogs, those in the present study averaging 0.55 mgm. per minute, as compared with other substances reabsorbed by the dog kidney, e.g., glucose, approximately 200–300 mgm. per minute (7, 8) and glycin amino nitrogen, 13–23 mgm. per minute (4). It is likely that the reabsorption of sodium chloride (9) and potassium salts (10) is preponderantly greater than that of ascorbic acid. Accordingly, it was assumed that in competition for any phase of the transfer mechanism, the ascorbic acid would be replaced by the salts; therefore, in the present study attention has been focused on the effects of these salts on the clearance of the vitamin.

EXPERIMENTAL PROCEDURE. Eight trained female dogs ranging in weight from 10.0 to 19.5 kgm. were used in various phases of this study. The dogs were kept on a diet of hospital scraps. Clearance experiments were conducted with constant intravenous infusion of creatinine and ascorbic acid in 0.9 per cent saline to maintain suitable plasma levels. Generally, collections were begun about 30 minutes after priming doses of these substances, to allow suitable time for equilibration. Urine was collected by catheter every ten minutes, and the bladder was washed out at the end of each period. The dogs were hydrated with 40 cc./kgm. of water prior to the experiment to insure adequate urine flows.

The clearance of creatinine was taken as the measure of the glomerular filtration rate. Creatinine content of the $CdSO_4$ (11) plasma filtrates and diluted

urines was measured by the alkaline-picrate method of Folin and Wu (12). Plasma and urinary ascorbic acid concentration was determined by the dichloroindophenol method with modifications given in the previous report (1). All analyses were done in duplicate. The tubular reabsorption of ascorbic acid was taken as the difference between the amount filtered, or *load* (glomerular filtration rate × plasma ascorbic acid) and the amount excreted (UV), in a minute's time. With the exceptions mentioned below, the load levels were considered adequate for complete tubular saturation.

The experiments were designed to fall into four categories: in the first, eighteen clearance experiments of from 5 to 8 periods in length were performed in 4 dogs in which only creatinine and ascorbic acid in 0.9 per cent saline were infused. These span in time the total length of experiments in which hypertonic salt was infused. They serve to show what the normal variations in ascorbic acid reabsorption are during the corresponding time interval in which salt was infused. In the second category appear six experiments in three dogs in which 20 per cent mannitol was infused at the rate of 1.1 cc. per minute after a priming dose of 5 grams of mannitol, following the establishment of the normal ascorbic acid reabsorptive Tm value in three control periods. These experiments were designed to show that the reabsorption of ascorbic acid is not influenced by the presence in the renal tubules of a substance of high osmotic activity, which would affect water reabsorption. Since mannitol is not reabsorbed by the tubules (13), it cannot itself compete with the ascorbic acid reabsorption. In the third category are nine experiments in two dogs in which after two or three control periods infusion of approximately 6 per cent NaCl solution at 1.1 cc./min. followed an average priming dose of 4.5 grams of NaCl. In the fourth category appear 6 experiments in two dogs in which following two control observations from 19 to 24 cc. of 10 per cent KCl were infused during a time interval of ten minutes. Because of the presumptive toxicity of the potassium ion, we have thus limited the time during which relatively high concentrations of the ion might act. Beyond occasional bradycardia, the dogs however showed no symptoms during the KCl injection.[1]

To exclude the possibility that alterations in the renal vascular supply might result from the infusion of the salts, and thus influence the filtration and reabsorption of the ascorbic acid, we have in six experiments in which KCl was given studied the effects on the clearance of *p*-aminohippuric acid, which was taken as the effective renal plasma flow (14). The results obtained with injections of KCl are taken to be typical of the salt effects, and we have chosen it in particular because of the higher rate of infusion, and the possibility of a vascular effect (e.g., bradycardia, with the possibility of decreased blood flow to the kidney).

[1] The amounts given on a per kilogram basis were as follows:

Mannitol: priming, av. 2.23 mM/kgm. (range: 2.03–2.74 mM/kgm.); infusion: av. 0.0933 mM./kgm./min. (range: 0.0824–0.1043 mM/kgm./min.).

Sodium chloride: priming, av. 4.11 mM/kgm. (range: 3.2–6.42 mM/kgm.); infusion: av. 0.0684 mM/kgm./min. (range: eight between 0.0514–0.071 mM/kgm./min., one at 0.133 mM/kgm./min.).

Potassium chloride: infusion at rate of 0.161 mM/kgm/min., for ten minutes.

RESULTS. A. *Effect of prolonged infusion of creatinine and ascorbic acid in isotonic saline on ascorbic acid reabsorption.* In figure 1 a total of 99 individual urine collection periods are presented in terms of the ratio: milligram reabsorbed per minute for each individual urine period, divided by the average reabsorption for the entire clearance experiment. In fourteen of these experiments the load/Tm ratio was adequate to insure complete tubular saturation, and the averate Tm values in mgm. per minute for the four dogs were: 0.50 (2 expts., 13 kgm. dog); 0.64 (2 expts., 16 kgm. dog); 0.75 (5 expts., 16 kgm. dog); and 0.625 (5 expts., 19 kgm. dog). Four other experiments which were done at load levels inadequate for tubular saturation were accepted because the load level remained

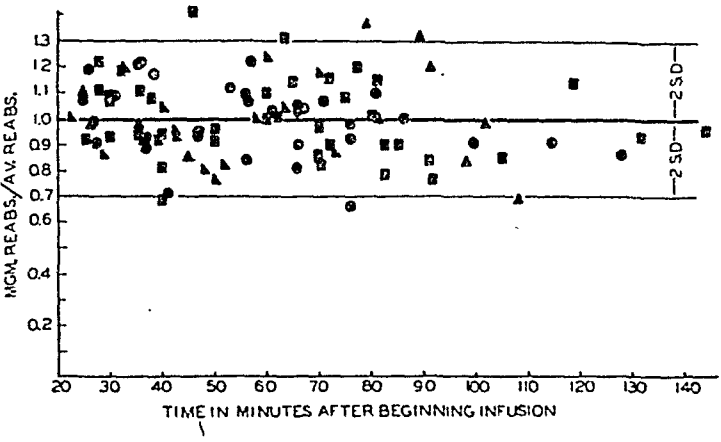

Fig. 1

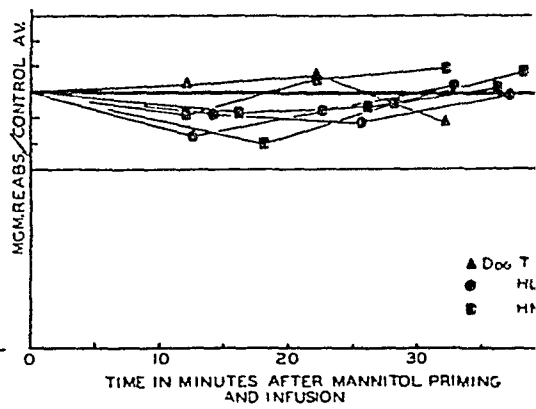

Fig. 2

Fig. 1. Showing the normal variation in the tubular reabsorption of ascorbic acid during prolonged infusion. Each point represents the value for an individual urine collection period expressed as the ratio to the average reabsorption for the entire clearance experiment. The data are derived from eighteen experiments of from five to eight periods each in four dogs. The limits ± 2 S.D. (standard deviation from the mean) serve to indicate the range of normal variation.

Fig. 2. Showing the effect of infusion of 20 per cent mannitol solution on the ascorbic acid reabsorptive Tm. The amount of reabsorption for each experimental period is expressed as the ratio to the average Tm of three control periods.

constant throughout the clearance, and hence did not influence the rate of reabsorption.

It is seen that over 90 per cent of the data fall within the limits of ± 2 S.D. (standard deviation from the mean). Accordingly the experimental data which follow are considered significant when they fall outside these limits. The data in figure 1 show that there appears to be no significant trend in ascorbic acid reabsorption as the result of prolonged infusion with 0.9 per cent saline for intervals as long as 150 min. The variations seen from period to period are probably the result of emptying and dead-space errors.

B. *Effect of mannitol.* (Table 1, part A; fig. 2.) Since the infusion of hypertonic NaCl and KCl may be followed by diuresis at least partly attributable to osmotic action in the tubules, it was conceivable that the resulting decrease in water reabsorption might influence the tubular reabsorption of ascorbic acid. Accordingly mannitol, which has been demonstrated to be an effective osmotic

TABLE 1

Effect of mannitol, sodium chloride, and potassium chloride on the clearance of ascorbic acid

DOG	CONTROL				EXPERIMENTAL PERIODS							
	No. periods	Av. Load*	Av. Tm:	CAA† / CCr	Av. Load	Reabs. per min. 1	2	3	Reabs./Tm 1	2	3	CAA / CCr
A. Mannitol												
		mgm./min.	*mgm./min.*		*mgm./min.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	
HL	3	1.842	0.368	0.800	1.814	0.305	0.342	0.380	0.83	0.93	1.03	0.817
12 kgm.	3	1.845	0.433	0.770	1.840	0.395	0.384	0.430	0.92	0.89	1.00	0.780
HN	3	1.255	0.444	0.647	1.548	0.410	0.420	0.455	0.92	0.95	1.03	0.726
13.5	3	2.500	0.492	0.803	2.825	0.450	0.520	0.540	0.91	1.06	1.09	0.819
kgm.	2	1.167	0.590	0.492	1.092	0.473	0.565	0.643	0.80	0.96	1.09	0.487
T 10 kgm.	3	1.453	0.500	0.657	1.580	0.510	0.530	0.440	1.03	1.07	.89	0.690
Average				0.695					0.90	0.98	1.02	0.720
B. Sodium chloride												
C	2	1.280	0.334	0.740	1.259	0.134	0.129	0.084	0.40	0.39	0.25	0.906
19.5	2	1.430	0.403	0.717	1.578	0.333	0.245	0.333	0.83	0.61	0.83	0.808
kgm.	2	1.550	0.473	0.694	1.707	0.193	0.201	0.293	0.41	0.43	0.62	0.866
	2	1.636	0.441	0.730	1.215	0.283	0.246	0.126	0.64	0.56	0.29	0.825
	2	1.762	0.435	0.755	1.717	0.000	0.000	0.000	0.00	0.00	0.00	1.030
R	3	1.105	0.393	0.643	1.340	0.217	0.234	0.227	0.55	0.59	0.58	0.831
16 kgm.	3	1.283	0.411	0.680	1.834	0.265	0.108	0.000	0.64	0.26	0.00	0.930
	3	1.480	0.610	0.577	1.993	0.310	0.240	0.195	0.51	0.39	0.32	0.878
	2	1.659	0.417	0.750	1.092	0.160	0.051	0.162	0.38	0.12	0.38	0.898
Average				0.700					0.48	0.37	0.36	0.885
C. Potassium chloride												
C	2	1.812	0.480	0.738	2.070	0.485	0.260	0.670	1.01	0.54	1.40	0.830‡
	2	1.821	0.650	0.645	1.643	0.135	0.522	0.475	0.21	0.80	0.73	0.929
	2	2.165	0.426	0.800	2.551	0.163	0.000	0.772	0.38	0.00	1.80	1.000
	2	2.455	0.860	0.647	2.341	0.727	0.325	0.903	0.85	0.38	1.05	0.870
R	2	1.545	0.483	0.687	1.147	0.038	0.000	0.479	0.08	0.00	0.99	1.000
	2	2.337	0.322	0.862	2.660	0.303	0.140	0.412	0.94	0.44	1.28	0.943
Average				0.730					0.58	.36	1.20	0.929

* Load: glom. filt. rate × plasma ascorbic acid.

† Ascorbic acid/creatinine clearance ratio.

‡ One period of maximum effect.

diuretic, was infused following three control periods. The results are given in table 1, and in figure 2 appear as the ratios of the amount reabsorbed during mannitol infusion over the average reabsorption of the control periods. Since no significant changes occurred in reabsorptive Tm, it can be concluded that the presence of an osmotically active substance in the tubules, although increasing the rate of the urine flow, did not influence the reabsorption of ascorbic acid.

C. *Effects of hypertonic NaCl.* (Table 1, part B; fig. 3.) With the continued infusion of hypertonic NaCl it was observed that the reabsorption of ascorbic acid was continually depressed. The ratios of each experimental period divided by the control reabsorption show the following average values: after 20 min.,

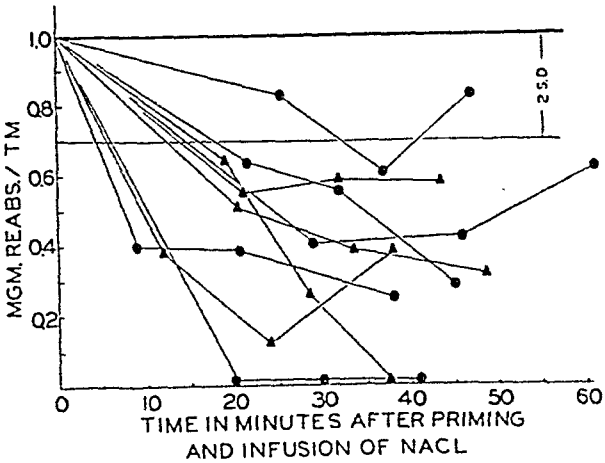

Fig. 3

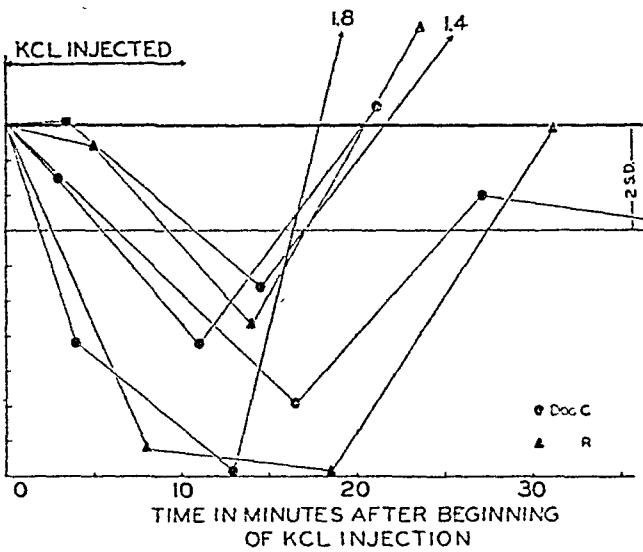

Fig. 4

Fig. 3. The effect of infusion of 6.0 per cent sodium chloride solution on the ascorbic acid reabsorptive Tm. The amount of reabsorption for each experimental period is expressed as the ratio to the average Tm of two or three control periods.

Fig. 4. The effect of infusion of 10.0 per cent potassium chloride solution on the ascorbic acid reabsorptive Tm. The experimental periods were taken during and after a ten minute period of infusion of potassium chloride solution, and the amount of reabsorption for each experimental period is given as the ratio to the average Tm of two control periods.

0.48 (−52 per cent); after 31.5 min., 0.37 (−63 per cent); after 44 min., 0.36 (−64 per cent). The decreased reabsorption resulted in an elevated clearance of ascorbic acid, the ascorbic acid/creatinine clearance ratio rising from 0.700 to 0.885.

D. *Effects of hypertonic KCl.* (Table 1, part C; fig. 4.) During the ten minute period of KCl infusion, the Tm of ascorbic acid was continually depressed and showed an average ratio to the control value of 0.58 (−42 per cent) 6.7 minutes after beginning of the infusion. At 16 minutes the lowest point was reached, 0.36 (−64 per cent), then rapidly returned to normal during the third period, at 27 minutes being 1.2. The rapid return to normal after cessation of the infusion was probably the result of the rapid clearance from the body of the potassium salt (10). During the second experimental period, when the

effect on reabsorption was maximal, the ascorbic acid/creatinine clearance ratio rose to 0.929 from a control average of 0.730.

E. *Effects of hypertonic KCl on glomerular filtration rate, effective renal plasma flow, and filtration fraction.* The results are summarized in table 2. Increases

TABLE 2

The effect of injection of potassium chloride on the glomerular filtration rate, effective renal plasma flow, and filtration fraction

	DOG	CONTROL*	DURING INJECTION				
			Periods			Av.	Change
			1	2	3		
		cc./min.				*cc./min.*	*per cent*
Glom. filt. rate: (creatinine UV/P)	C	86.5	88.0	102.0	97.3	95.7	+10.6
		58.0	74.5	49.4	75.5	66.5	+14.7
		67.0	75.4	74.3	88.0	80.0	+19.4
	R	64.0	66.3	74.1	105.3	82.0	+28.1
		54.5	57.6	52.1	62.0	57.2	+5.0
		60.0	69.0	78.0	71.0	72.7	+21.0
Average							+16.5
Renal plasma flow: (p-aminohippuric UV/P)	C	351	357.0	381.0	350.0	362.0	+3.14
		200	229.0	165.0	267.5	220.5	+10.25
		269	305.0	263.5	312.0	293.5	+9.10
	R	210	231.0	261.0	363.0	285.0	+35.70
		175	178.0	171.0	210.0	187.0	+6.85
		202	216.0	249.0	236.0	233.0	+15.35
Average							+13.4
Filtration fraction†	C	0.247	0.249	0.268	0.278	0.265	+7.30
		0.346	0.318	0.337	0.335	0.330	−4.63
		0.247	0.247	0.282	0.282	0.270	+9.30
	R	0.303	0.287	0.284	0.290	0.288	−4.95
		0.312	0.323	0.304	0.294	0.307	−1.60
		0.295	0.319	0.312	0.301	0.311	+5.43
Average							+1.81

* Average of 2 periods.

† $\frac{\text{creatinine clearance}}{\text{p-aminohippuric acid clearance}}$.

which average 16.5 per cent are seen in the glomerular filtration rate. These compare with an average increase of 12 per cent in filtration rate following the infusion of hypertonic NaCl, and are probably the result of plasma dilution following the action of the salts on the intracellular compartment. Corresponding increases (average +13.6 per cent) were noted in the *p*-aminohippuric acid clearances following KCl infusion, and as a result the filtration fraction showed

no significant changes. The conclusion is drawn that the injection of KCl produced no alteration in the blood supply to the kidney which might conceivably impede the rate of ascorbic acid filtration and reabsorption.

DISCUSSION. It has been demonstrated that the velocity of urine flow, as influenced by osmotic diuresis, does not affect the tubular reabsorption of ascorbic acid. Furthermore, the infusion of hypertonic salt does not decrease the rate of glomerular filtration and the renal plasma flow, indicating that the effects seen could not have resulted from a decrease in the ascorbic acid load to the reabsorptive mechanism, or from inefficient removal of the substance from the peritubular vascular bed. The conclusion follows that sodium and potassium chloride compete or interfere with the ascorbic acid transfer mechanism in some manner. Shannon (3) by elevation of the plasma glucose levels in dogs has completely blocked the tubular reabsorption of xylose, and has argued on this basis that xylose is actively reabsorbed by the same mechanism as glucose. Pitts (4) by completely blocking the reabsorption of creatine by elevation of plasma glycin has similarly concluded that a common transport mechanism exists for these substances.

Although several urine periods occurred in which the reabsorption of ascorbic acid was completely blocked by the salt, the reabsorption of sodium and potassium chloride and ascorbic acid have not been found to be as precisely related as the aforementioned substances. In view of the fact that the simultaneous clearance of the salts was not evaluated in this study, it cannot be said at this time what the exact nature of the relationship between the transfer of the vitamin and the salts is. It was noted, however, that the degree of ascorbic acid reabsorption was not consistently related to either the rate of infusion or the total dose of salt given. From this it appears that sodium and potassium chloride compete with the ascorbic acid transfer in some non-specific manner. Rather than occupying a common reabsorptive mechanism, these substances may share some general phase of the energetics of tubular transport.

SUMMARY AND CONCLUSIONS

1. A study has been made of the effects of the infusion of hypertonic sodium and potassium chloride solutions on the reabsorption of ascorbic acid by the renal tubules.

2. It has been found that the reabsorptive Tm is significantly depressed by these salts, showing that they block the ascorbic acid reabsorptive mechanism in some manner.

3. Possible interfering factors were not found to influence the filtration and reabsorption of ascorbic acid, e.g., increase in the rate of urine flow as the result of an osmotic diuretic action by a substance not itself reabsorbed by the tubules (mannitol), and the observation that no significant changes occurred in glomerular filtration rate and renal plasma flow during salt infusion.

4. The evidence does not indicate that ascorbic acid and the salts are reabsorbed by a common mechanism as in the case of glucose and xylose, and glycin and creatine, but that rather the salts impair the reabsorptive activity of the

tubules for ascorbic acid in some non-specific manner, the nature of which is unknown.

The authors wish to thank Dr. Homer W. Smith for his helpful criticism of the manuscript.

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THE MECHANISM OF SUCROSE DAMAGE OF THE KIDNEY TUBULES[1]

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Received for publication February 25, 1944

The exact mechanism by which sucrose injures the kidney tubules has not been established, and was the object of this investigation. Helmholtz in 1933 first reported that large doses of hypertonic sucrose given intravenously to rabbits provoked a severe hydropic degeneration limited to the proximal convoluted tubules and the ascending loop of Henle. A human autopsy case with identical lesions attributable to sucrose was also presented by Helmholtz, and this has been confirmed in a number of subsequent autopsy reports by Cutler (1939), Anderson and Bethea (1940), Anderson (1941), and Rigdon and Cardwell (1942).

Most tubular poisons, like sucrose, act selectively upon the proximal convoluted tubules, and only slightly damage the distal convoluted segments. In this study sucrose was administered to rabbits with unilateral hydronephrosis in which the obstructed kidney cannot elaborate a concentrated urine, to phlorizinized rabbits and rats in which sucrose reabsorption is blocked, and to aglomerular toadfish which cannot secrete sugar, and the kidneys in each case were examined histologically.

Experimental observations. A standard intravenous dose of sucrose which would produce a maximum hydropic degeneration was determined. A series of 11 normal rabbits weighing 1.4 to 4.1 kilos were given from 70 to 320 cc. of 20 per cent sucrose (sterile ampules, Lilly). The various doses ranged from 7.8 to 20 grams of sucrose per kilo. Following the first injection, or the second injection if administered in a divided dose, the animals were allowed to live for from 2 to 72 hours, with the exception of one rabbit which was sacrificed after 30 days. Only this latter rabbit failed to reveal some hydropic degeneration. It was found that when 40 cc. of 20 per cent sucrose per kilo was given intravenously, followed in 24 hours by a second equal dose, a uniformly severe hydropic degeneration was present 24 hours after the second injection. This, then, was our standard dose. All tissues were fixed in Helly's solution (bichromate formalin mixture).

One rabbit which received half the standard dose, i.e., only the first injection, but was not allowed to drink water subsequently showed a severe hydropic degeneration after 24 hours. Five cubic centimeters of 50 per cent sucrose was injected intraperitoneally in each of 2 rats that were not allowed to drink water during the following 24 hours. Similarly 2 rabbits were administered 40 cc. of 50 per cent sucrose, and one rabbit 50 cc. of 20 per cent sucrose intraperitoneally. Both rats and the three rabbits showed the classic tubular hydropic picture.

[1] Submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy in the Graduate School of the University of Minnesota.

These experiments show that when water is withheld, and the urine is more concentrated, a smaller dose of sucrose is effective in producing injury.

The left ureter of 10 rabbits was doubly ligated and severed about 1.5 cm. below the uretero-pelvic junction. The animals were anesthetized with intravenous sodium pentothal, and surgery carried out with aseptic technique. After periods of hydronephrosis of from 2 to 31 days the standard intravenous dose of sucrose was administered. This produced severe hydropic degeneration in both the unoperated and the hydronephrotic kidneys at all intervals studied (table 1).

Two rabbits and 21 rats were phlorizinized and then given sucrose injections which had resulted in uniform, severe hydropic injury in normal control animals. Two per cent phlorizin in olive oil was employed, and the animals starved during the period of phlorizination. The first rabbit received a daily subcutaneous injection of 300 mgm. of phlorizin per kilo for 6 days. On the fifth day this animal was given 100 cc. of 20 per cent sucrose intravenously, but during the

TABLE 1

Nephrotoxic effect of intravenous sucrose in the presence of unilateral hydronephrosis

RABBIT EXPERIMENT NO.	DURATION OF HYDRONEPHROSIS BEFORE INJECTION	TOTAL DOSE 20 PER CENT SUCROSE*	KIDNEY HYDROPIC DEGENERATION	
			Right (unoperated)	Left (hydronephrotic)
	days	*cc.*		
R-12	2	260	++++	++++
R-13	3	230	++++	++++
R-14	5	250	++++	++++
R-15	6	250	++++	++++
R-16	11	270	++++	++++
R-17	14	250	++++	++++
R-18	19	208	++++	++++
R-19	31	120†	++	++

* Standard dose.

† Rabbit died after receiving one injection, or half standard dose.

second injection the following day the rabbit died. Nevertheless, sucrose had caused 2 plus hydropic tubular damage. The left ureter of the other rabbit was doubly ligated and severed two weeks prior to phlorizination; the animal received 250 mgm. of phlorizin per kilo per day for 6 days and the standard intravenous dose of sucrose. Despite complete glycuresis induced by phlorizin, 4 plus hydropic degeneration was produced (table 2).

A series of 12 rats weighing from 200 to 240 grams were phlorizinized with 20 to 25 mgm. of phlorizin in olive oil, subcutaneously, per day for six days. Nine of these rats were given an intraperitoneal injection of sucrose, while three controls were not. A series of 9 rats were acutely phlorizinized by administering 10 mgm. of a 1 per cent solution of phlorizin freshly prepared in 2 per cent aqueous sodium bicarbonate. At the same time they were given, subcutaneously, 20 mgm. of 2 per cent phlorizin in olive oil. Eight of these rats were also given 5 cc. of 50 per cent sucrose intraperitoneally immediately following the phlorizin.

One control rat was not given sucrose. The animals were allowed neither food nor water and killed in 24 hours. Despite phlorizin, sucrose produced severe hydropic degeneration.

As a control for the nephrotoxic properties of sucrose, xylose, inulin and dextrose were administered to rats and rabbits. Five normal rabbits and three normal rats received 20 per cent dextrose in amounts equivalent to the sucrose dose which had provoked hydropic injury. Under ether anesthesia the left ureters of three rats were doubly ligated and severed. Seven days thereafter,

TABLE 2

*Nephrotoxic effect of sucrose in phlorizinized animals**

ANIMAL EXPERIMENT NO.	METHOD OF PHLORIZINATION	TOTAL DOSE OF PHLORIZIN	DOSE AND ROUTE OF SUCROSE ADMINISTRATION	KIDNEY HYDROPIC DEGENERATION
		grams		
R-28	Chronic	3.50	100 cc. 20 per cent intravenous	++
R-29	Chronic	3.50	200 cc. 20 per cent intravenous	++++† ++++‡
Rats 3–9	Chronic	0.12	5 cc. 50 per cent intraperitoneal	++++
Rat 10	Chronic	0.12	None (control)	0
Rats 11, 12	Chronic	0.20	5 cc. 50 per cent intraperitoneal	++++
Rats 13, 14	Chronic	0.20	None (controls)	0
Rats 15–22	Acute	0.02 0.04	5 cc. 50 per cent intraperitoneal	++++
Rat 23	Acute	0.40	None (control)	0

* Phlorizin for these experiments was kindly given to us by Dr. Harold C. Hodge of the School of Medicine and Dentistry of the University of Rochester, and by Dr. R. H. Barnes of the University of Minnesota Medical School.

† Unoperated.

‡ Hydronephrotic.

7 cc. of 50 per cent xylose was administered intraperitoneally in two of these animals; the other received by the same route 10 cc. of 20 per cent inulin freshly prepared in 0.6 per cent sodium chloride at 85°C. In confirmation of previous work, dextrose was not found to injure the tubules. Likewise, xylose and inulin did not produce hydropic degeneration (table 3).

Three rats, pregnant for 18 days, received 5 cc. of 50 per cent sucrose intraperitoneally. One rabbit, pregnant for 14 days, was administered the standard intravenous dose. The maternal rat kidneys revealed 3 plus hydropic degenera-

tion, but the fetal kidneys were unaffected. On the other hand, both the maternal and the fetal rabbit kidneys showed 4 plus hydropic degeneration. We have no explanation for this apparent discrepancy.

Five toadfish—marine teleosts, *Opsanus tau*—were subjected to intraperitoneal injections of 25 and 50 per cent sucrose in divided doses. One half of the total dose was given at the time the fish were removed from the common aquarium tank and placed in individual tanks. The second half was injected 24 hours later, and the fish killed 24 hours thereafter.[2] Sucrose did not induce hydropic degeneration in the aglomerular tubules of the common toadfish (table 4). These fish, weighing from 510 to 750 grams had been kept in cap-

TABLE 3

Failure to demonstrate hydropic degeneration following administration of dextrose, xylose and inulin

ANIMAL EXPERIMENT NO.	DRUG	DOSE AND ROUTE OF ADMINISTRATION	DURATION OF LIFE AFTER INJECTION	KIDNEY HYDROPIC DEGENERATION
			hours	
R-30	Dextrose	100 cc. 20 per cent intravenous	Immediate death	0
R-31	Dextrose	180 cc. 20 per cent intravenous	1	0
R-32	Dextrose	320 cc. 20 per cent intravenous*	24	Granular swelling
R-33	Dextrose	120 cc. 20 per cent intravenous	20	0
R-34	Dextrose	200 cc. 20 per cent intravenous	72	0
Rats 24–26	Dextrose	4 cc. 50 per cent intraperitoneal	24	0
Rats 27, 28	Xylose	7 cc. 50 per cent intraperitoneal	24	0†
Rat 29	Inulin	10 cc. 20 per cent intraperitoneal	24	0†

* Dextrose given in divided dose comparable to standard sucrose dose.

† Unilateral hydronephrosis established 7 days before injection, neither kidney affected.

tivity for several years in the John G. Shedd Aquarium in Chicago. The aquarium water, assumed to be relatively constant, was tested at the time of our experiments: pH 8.2, specific gravity 1.025, temperature 60–61°F.

As controls, one normal toadfish was killed and one toadfish was given 20 cc. of 50 per cent dextrose intraperitoneally. Two goldfish, weighing 20 and 32 grams, were administered a single intraperitoneal injection of 20 per cent sucrose. The glomerular tubules of the goldfish showed the characteristic hydropic effect (table 4).

Discussion. The hydropic degeneration from sucrose, as described by

[2] Mr. W. H. Brunskill of the Shedd Aquarium staff rendered valuable assistance at the aquarium where the experiments were carried out.

numerous observers, consists of a finely reticular cytoplasm, foamy with vacuolation, and resembling the pathologic hypernephroma cell (figs. 1 and 2). Often the tubular lumen is closed, but in spite of this there is a diuresis. Perhaps there is some distortion in fixation. Pyknosis and karyolysis are demonstrable. Unless large doses of sucrose are administered (Helmholtz, 1933), or the animals dehydrated (Rigdon and Cardwell, 1942) the hydropic degeneration is patchy in distribution and cellular swelling only moderate. Severe hydropic degeneration may be noted within one or two hours following massive doses of hypertonic sucrose but after 15 days the tubules appear normal (Helmholtz, 1933).

Following the injection of sucrose in man, Keith, Power and Pederson (1934) were able to recover the sugar quantitatively from the urine within two hours. In the dog, however, they were able to recover only 70 to 80 per cent of the quantity injected.*

TABLE 4

Nephrotoxic effect of sucrose on toadfish and goldfish kidneys

FISH EXPERIMENT NO.	INTRAPERITONEAL DOSE OF SUCROSE	DURATION OF LIFE AFTER LAST INJECTION	KIDNEY HYDROPIC DEGENERATION
		hours	
Toadfish-1	30 cc. 50 per cent*	24	0
Toadfish-2	20 cc. 50 per cent*	24	0
Toadfish-3	20 cc. 50 per cent*	24	0
Toadfish-4	44 cc. 25 per cent*	24	0
Toadfish-5	44 cc. 25 per cent*	24	0
Toadfish-6	(Control) 20 cc. 50 per cent dextrose	24	0
Toadfish-7	(Control) no sugar		0
Goldfish-1	1 cc. 25 per cent	24	+++
Goldfish-2	1 cc. 25 per cent	24	+++

* Total dose given in 2 equal injections 24 hours apart.

Because sucrose causes hydropic degeneration more readily in dehydrated subjects, Anderson (1941) suggested that reduced glomerular filtration enhances the damage by prolonging the exposure of the tubular cells to sucrose. Yet we found that when less than the standard dose of sucrose is administered to a rabbit with unilateral hydronephrosis (rabbit 19) the hydropic degeneration is of the same intensity in the hydronephrotic kidney where filtration is probably reduced as in the unoperated kidney. When the standard dose of sucrose was administered to rabbits with unilateral hydronephrosis of varying intervals of duration up to one month, it produced equally severe damage in both kidneys (figs. 1 and 2). In this respect sucrose differs from uranium nitrate and bichloride of mercury which do not injure the hydronephrotic kidney (Wilmer, 1943).

Since sucrose injures the tubules when the glomerular filtrate is not concentrated (hydronephrosis), we may assume that the nephrotoxic action occurs at a critical plasma or glomerular filtrate level. Since the sugar is quantitatively

eliminated within a few hours the injury is probably induced within this short interval. Because injury is more severe and persistent following divided doses (Helmholtz, 1933), there is probably a summation of acute insults.

Sucrose bathes the tubule cells from both the surrounding rich vascular network and from the lumen. Therefore, it was necessary to determine whether or not the sugar injures the cells from the vascular or from the luminal side.

To prevent sucrose from entering the lumen of the tubule it was necessary to resort to the aglomerular fish which cannot secrete sugars. Through the kindness of Walter H. Chute, Director of the John G. Shedd Aquarium in Chicago,

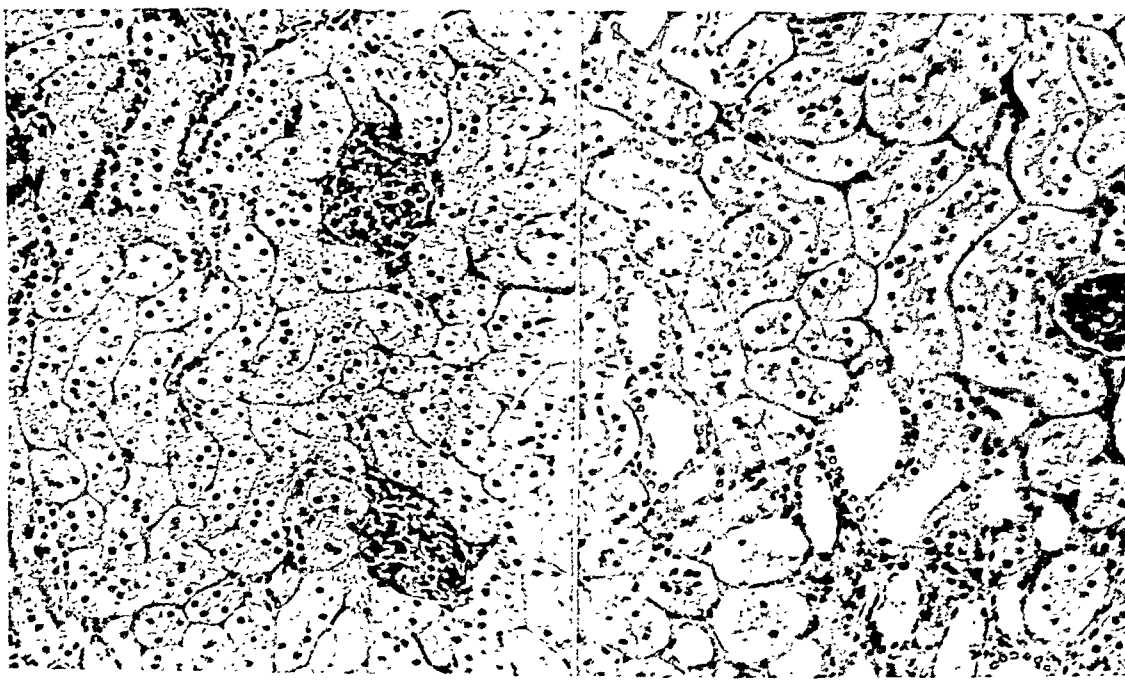

Fig. 1 Fig. 2

Fig. 1. Unoperated right kidney of rabbit R-16 given standard intravenous dose of sucrose resulting in 4 plus hydropic degeneration. Photomicrograph × 160.

Fig. 2. Hydronephrotic left kidney of rabbit R-16 revealing equally intense hydropic degeneration. Photomicrograph × 175.

we were given seven toadfish and the aquarium facilities to carry out these experiments.

The entire function of the toadfish kidney is performed by a single tubular segment composed entirely of one type of cell corresponding to that of the proximal convoluted tubular epithelium of the mammalian organ (Defrise, 1932). A fundamental difference between the glomerular and aglomerular kidneys is the inability of the aglomerular kidney to eliminate glucose and other sugars including sucrose (Marshall, 1934). The impermeability of the toadfish kidney to glucose is a relative matter since traces of glucose appear in the urine after phlorizin injections and very high blood sugar levels; this trace is comparable to that normally found in human urine which is ordinarily considered to be free

of sugar (Marshall, 1930). Considering the fact that the enzyme phosphatase probably plays a rôle in the transport of the glucose molecule through the tubule cells, it is interesting to note that the toadfish is the only animal whose tubules are known to be devoid of this enzyme (Wilmer, 1944).

Clarke (1935) injected large quantities of sucrose intravenously in the aglomerular goosefish, *Lophius piscatorius*, and obtained plasma levels of 540 and 1060 mgm. per 100 cc. During the following four hours the urine contained less than 1 per cent sucrose. Not only does this show that sucrose is not secreted by the aglomerular tubule, but that diffusion across the cells is so small as to be entirely negligible (Smith, 1937).

Five toadfish were each given two intraperitoneal injections of sucrose. Their kidneys did not reveal any evidence of hydropic degeneration, and were indistinguishable from the normal control and the control fish given dextrose. Although sucrose has been given to aglomerular fish previously, we were unable to find any description of the renal histology following the administration of the sugar. Contrary to our findings in the toadfish, goldfish, which had been given a single intraperitoneal injection of sucrose, were found to have severe hydropic degeneration. This seems to be good evidence that sucrose is not nephrotoxic from the blood stream side of the tubules alone. The toadfish at our disposal had been kept in captivity for several years and these experiments should be confirmed with freshly caught fish.

Since sucrose does not injure the tubules from the vascular side alone, it probably does so from the lumen. The sugar is absorbed from the tubule since the sucrose clearance is the same as that of xylose and about 20 per cent less than inulin which is considered not to be reabsorbed at all (Smith, 1937). In phlorizinized man and animals the sucrose clearance is the same as inulin clearance, hence phlorizin blocks sucrose reabsorption. As little as 2 per cent of the inulin in the glomerular filtrate diffuses back, while the diffusion coefficient of sucrose is about three times as great (Bunim, Smith and Smith, 1937), and it would seem that at least 6 per cent of it diffuses back. To prevent the reabsorption of sucrose, a series of rats and rabbits were phlorizinized. Sucrose, nevertheless, caused severe hydropic degeneration in these animals. This appeared to eliminate tubular reabsorption of sucrose as necessary for the production of hydropic injury. Steinitz (1940) reported that, contrary to the prevailing opinion, sucrose and inulin clearance are identical in the human subject. If such is the case, sucrose injury is probably induced in man by diffusion, as in our phlorizin animal experiments.

Phlorizin probably does not interfere with the physical phenomenon of diffusion, so that the sucrose which diffuses through the cells accounts for the hydropic degeneration. It is possible that diffusion may occur from both the vascular and luminal side; no experiment comes to mind which would eliminate diffusion. Since sucrose is practically excluded from cells, its diffusion into the tubular epithelium places it in an abnormal intracellular situation, and probably causes an osmotic disturbance with the resultant hydropic type of damage. The fact that the tubules quickly recover, while the hydropic degeneration due to poisons

like racemic tartaric acid (Underhill, Wells and Goldschmidt, 1913), and diethylene glycol (Geiling and Cannon, 1938) proceed to necrosis, argues that it is perhaps a primary osmotic effect, and not the result of cellular membrane damage, hygroscopic action of the drug, or primary toxic injury. One must assume in addition some specific tubular susceptability to sucrose.

SUMMARY

Sucrose administered intravenously in large doses produces a severe hydropic degeneration in both the unoperated and hydronephrotic kidneys of the rabbit. Since the hydronephrotic kidney cannot elaborate a concentrated urine, a critical plasma level is probably essential for the nephrotoxic effect.

Aglomerular toadfish, *Opsanus tau*, which cannot secrete sucrose were given the sugar intraperitoneally without nephrotoxic effect. Therefore, it would seem that sucrose does not injure the tubule from the vascular side when simultaneously excluded from the lumen. Sucrose provokes hydropic degeneration in the glomerular tubules of the goldfish.

When reabsorption of sucrose is blocked in phlorizinized rabbits and rats this sugar still produces its characteristic hydropic degeneration. Hence, sucrose reabsorption is not necessary for the toxic injury.

The small amount of sucrose which diffuses into the tubule cells, even in the phlorizinized animals, is probably the mechanism by which this sugar injures the kidney.

Acknowledgment. I am greatly indebted to Dr. E. T. Bell for suggesting this study and for indispensable advice and criticism.

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* In a paper by Keith and Power (1937) which I had not seen, it was reported that 89 to 98% of injected sucrose was excreted in 10 to 12 hours. Although a considerable portion was eliminated in two hours, some remained in the body longer.

THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 141 JUNE 1, 1944 No. 4

OBSERVATIONS ON THE BEHAVIOR AND NEUROPHYSIOLOGY OF ACUTE THIAMIN DEFICIENT CATS[1]

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Received for publication March 16, 1944

The nervous manifestations of thiamin deficiency may be divided into acute reversible functional disorders and chronic degenerative lesions. The latter group have received the most attention with some attempt to correlate the nerve tissue damage with the functional disorders observed. Swank (1940) studied the degenerative changes which occur in the peripheral nerves of chronic thiamin deficient pigeons. The extent of the lesion paralleled the severity of paralysis at the time the bird was killed. From a detailed histological examination of the peripheral and central nervous system of chronic deficient rats, Prickett (1934) found no abnormal changes in peripheral nerves, but observed lesions in the medulla and pons. No paralysis was seen in these rats, but marked ataxia and disturbances of posture were present. At best such studies can tell little about the reversible functional disorders where actual tissue damage is ruled out by the rapid recovery when thiamin is administered.

Brief descriptions of the acute signs of thiamin deficiency have been reported for a number of species. In all cases anorexia is the first evidence of deficiency. After three to four weeks on diet, ataxia and convulsions suddenly appear in the rat (Prickett, 1934), the cat (Odom and McEachern, 1942), and pigeon (Swank, 1940). The dog shows ataxia and spasticity of the extremities but does not exhibit convulsive behavior (Swank, Porter and Yeomans, 1941). Apparently the only work which goes beyond a description of general behavior in thiamin deficiency is that of Church (1935), who studied reflex activities in deficient rats and found changes indicative of functional disorders in the brain stem.

The present paper reports a similar but more extensive study of behavior and reflex activities in acute thiamin deficient cats.

I. *Development of specific thiamin deficiency.* One of the weakest points in

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much of the physiological and biochemical work on thiamin deficient animals has been the inadequate control of vitamin supplements, particularly other members of the B-complex group. In the older studies the diets have been fortified with autoclaved brewer's yeast which supposedly contains sufficient quantities of the B-complex vitamins other than thiamin. However, the possible destruction of other vitamins in the autoclaving process, particularly thermolabile pyridoxine and pantothenic acid, cannot be excluded. A further criticism arises from the anorexia which gradually develops in thiamin deficient animals and greatly increases the difficulty of supplying adequate vitamin intake if only the addition of autoclaved yeast is relied upon. Forced feeding is not tenable as it usually produces vomiting in such animals. From these considerations the possibility of multiple deficiencies arising with such dietary regimes cannot be easily dismissed. To avoid these criticisms the more recent experiments have used supplements of crystalline vitamins, administered either orally or parenterally, independent of the diet.

Materials and dietary regime. The present studies were made on a group of 50 cats. Only adult males in good health and nutritional state were selected. Their weights ranged from 2.7 to 4.5 kgm. The animals were kept in individual cages in a steam heated room at an average temperature of approximately 25°C. The cats were weighed and examined once a day, usually in the late afternoon just before feeding.

The chief component of the diet consisted of a canned dog food[3] containing ground rabbit meat and bone, wheat, rolled oats, soy bean flour and carrots. Charcoal and cod liver oil were added. The analysis of this food as given by the manufacturer is: crude protein (min.) 12 per cent; crude fat (min.) 3 per cent; crude fiber (max.) 2 per cent. Thiamin was destroyed by autoclaving the sealed cans at 15 pounds pressure for five hours. Under the same conditions brewer's yeast was also autoclaved in open glass dishes. A solution containing pyridoxine hydrochloride, riboflavin and calcium pantothenate[4] was prepared fresh every week and kept in the refrigerator at 5°C. The completed diet was mixed fresh each day and has the following composition:

Amounts per cat per day

Autoclaved canned dog food	225 grams
Autoclaved brewer's yeast	0.5 gram
Pyridoxine HCl	0.4 mgm.
Calcium pantothenate	1.0 mgm.
Riboflavin	0.5 mgm.

Once a week 5 cc. of cod liver oil were added. Since the vitamin requirements of the cat have not been determined the quantities of crystalline vitamin supplements are arbitrary and represent approximately double the requirements determined for the dog by Schafer et al. (1941).

[3] Wild-Life Rabbit Meat Dog Food prepared by General Laboratories, Inc., Des Moines, Iowa.

[4] We wish to thank Dr. D. F. Robertson and Mr. L. J. Ruland of the Merck Company for generous supplies of crystalline vitamins.

Once a week the animals on diet were injected intraperitoneally with the same quantities of crystalline vitamins as given in the food. Also, 5 mgm. of alpha tochopherol were given orally or injected.

As anorexia developed the amount of canned food was reduced proportionately while the yeast and other supplements remained unchanged.

II. *Experimental evidence for specific thiamin deficiency.* In figure 1, curve *A*, is shown the typical gradual weight loss which occurs in cats on thiamin deficient diet. The loss per day was greatest in the second week and then became fairly constant, a decrease of 30 to 50 grams a day being found in most cases. The total weight loss in 75 per cent of the animals studied was of the order of 20 to 30 per cent of the original body weight. About 10 per cent of the cats showed

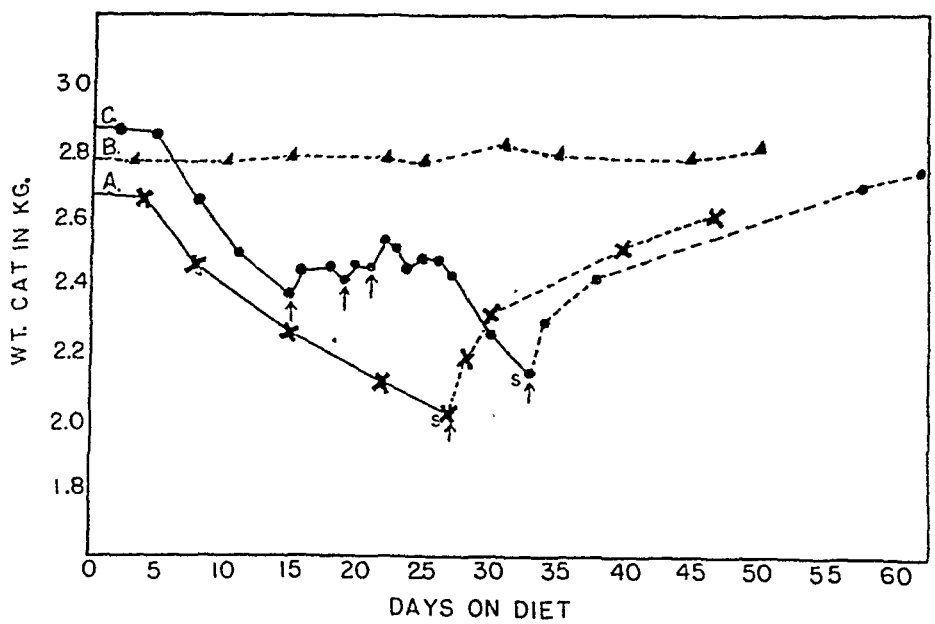

Fig. 1. Weight curves of cats. Solid line, thiamin deficient diet. Broken line, diet plus 0.5 mgm. of thiamin per day.

Curve *A*, cat 10 on diet. Convulsive seizures appeared at *s*.

Curve *B*, cat 3 on diet plus thiamin.

Curve *C*, cat 7 on diet. Single injections of 0.5 mgm. thiamin at arrows.

decreases of only 10 per cent. In another 15 per cent weight losses of 35 per cent or more were found. The only other obvious signs of deficiency during this first period, lasting about three weeks, were anorexia and vomiting. Slight ataxia was observed in the hind limbs during the third and fourth week, inaugurating the onset of the critical stage characterized by abnormal posture, ataxia, asynergia, dysmetria and short tonic convulsive seizures. The critical stage usually appeared in 25 to 35 days from the beginning of the diet. Prostration and death followed a few days later.

To check the adequacy of the diet a series of cats were placed on the thiamin deficient regime with the addition of 0.5 mgm. of thiamin per day. A typical case is shown in figure 1, curve *B*. In every instance the animal maintained its original body weight or gained, and showed no neurological disorders after six

weeks to three months on diet. Thus, the diet was complete except for thiamin if judged by its ability to maintain the animals in good health.

The anorexia and concomitant weight loss were clearly the result of thiamin deficiency as shown by the following experiment. A group of animals on diet, allowed to develop anorexia, were injected with 0.5 mgm. of thiamin intramuscularly. Appetite returned within an hour and with no further thiamin the animals ate normally for a few days and then gradually developed anorexia again. A case typical of the whole series is shown in figure 1, curve *C*. Each time thiamin was injected the animal's weight increased due to the increased food consumption and possibly better utilization, after which the weight curve levels off and falls with the decline of appetite. Essentially similar results were obtained when the thiamin was given orally.

The convulsive seizures and other neurological disorders which appeared in the critical stage were also clearly the result of thiamin deficiency. In 40 convulsive cats the injection of 1.0 mgm. of thiamin brought about a rapid, dramatic recovery. Convulsions disappeared within an hour and the following day the animal was essentially normal. The injection of calcium pantothenate, pyridoxine HCl, riboflavin, alpha tochopherol, choline, nicotinic acid, into critical stage animals brought about no improvement, the animal eventually dying if thiamin was not given. The administration of glucose likewise showed no curative action. Particular attention was given in these studies to supplying adequate pyridoxine and pantothenic acid because a deficiency of these vitamins may give rise to disorders somewhat similar to those found in thiamin deficiency. Wintrobe, Stein, Miller, Follis, Najjar and Humphreys (1942) have reported epileptiform seizures in pyridoxine deficient pigs and also nervous disorders due to pantothenic acid deficiency. In the same animal on thiamin deficient diet no neurological signs other than anorexia and vomiting were observed (Wintrobe et al., 1942). In the cat, however, the situation is clearly different for the dietary regime used, and the experiments reported here seem to limit the observed effects strictly to a specific lack of thiamin. Since the course of thiamin deficiency in the present studies developed rapidly and ended with death in a period of 30 to 40 days, it may be properly considered an acute deficiency in distinction to the chronic type studied by other investigators in animals where symptoms appear after 60 to 200 days on diet.

III. *General behavior.* The description which follows is a composite of the salient features which were most often observed in the series of 50 cats studied. The course of thiamin deficiency in the cat may be considered to occur in three stages.

1. Induction stage. The first change in the behavior of the thiamin deficient cat is a gradual loss of appetite which usually begins in the second week on diet, with complete anorexia by the fourth week. Vomiting may also occur during this period. Other than the decline in food consumption the general behavior of the animal is normal. It shows interest in food and salivates, but little or nothing is actually eaten. Walking is normal until the end of the third or fourth week, when a slight ataxia is often present in the hind limbs. Within a day the

animal passes from this period of essentially normal behavior into the critical stage.

2. Critical stage. This is characterized by a host of nervous disorders. Abnormal posture, ataxia and pupil dilatation are constant features, with short tonic convulsive seizures in 80 per cent of the cases. During this period the cat walks on a broad base with an ataxic, swaying gait. Walking in small circles is commonly observed. In most cases the head is markedly ventroflexed at all times and may show a twist to one side. On the second and third day the functional disorders become progressively more severe.

When present, the most striking episodes of the critical stage are sudden convulsive seizures which may occur spontaneously at any time in the walking or resting animal. There are no preliminary signs. Suddenly the head and neck ventroflex maximally, the fore and hind limbs are extended and abducted, and the body tends to flex. Such tonic seizures last from 3 to 5 seconds and terminate as suddenly as they begin. In a few cases convulsions lasting somewhat longer were observed, with both tonic and clonic components present.

Pupillary dilatation is always maximal during and immediately following seizures.

The early period of the critical stage is often associated with considerable general activity of the animal. There seems to be little or no impairment of higher cortical functions as judged from general behavior. The cat still shows interest in food, will come to the observer when called, and will follow. When the animal is picked up, marked spastic tone in the limb and body muscles is noted. Limb abduction and powerful movements of the trunk are commonly observed in carrying such animals. Cats in the critical stage are unable to jump from one level to another. The head and neck ventroflex and the animal turns half a somersault, landing on its back.

It has been mentioned that seizures appear spontaneously in the resting animal. They may also be precipitated by external stimuli such as loud noises or bright lights. By far the most effective method is to suddenly change the animal's orientation or position. Thus, holding the cat supine and then placing it back on its feet will almost invariably produce a seizure.

The marked neurological disturbances observed in the early period of the critical stage must be considered reversible functional disorders since all signs of the deficiency disappear within a day following the injection of thiamin. Cats given thiamin during the late critical stage may show some functional impairment, particularly ataxia in the hind limbs, for several weeks.

3. Terminal stage. The activities of the critical stage gradually lessen until the animal becomes weak and unable to walk. At this time it enters the terminal stage. In some cases extensor tone appears in the limbs and the animals seem comatose.

All attempts to recover animals from the terminal stage with thiamin failed. It is therefore considered a period of irreversible changes which lead to death within a day or two. The period from the onset of the terminal stage to death is 1 to 3 days.

IV. *Study of reflex action in thiamin deficient cats.* The present series of experiments on the reflex activities of cats in the critical stage were conducted with the idea of localizing more precisely the site of the functional nervous disorders observed. Extensive use has been made here of the monograph by Fulton (1938) which contains an admirable summary of the reflex activities of the cat and the neural pathways involved.

1. Spinal reflexes. The flexor withdrawal of the limb from painful stimuli was present in all stages. The knee jerk was also essentially normal.

2. Postural reflexes. The proper maintenance of orientation of the head and body when an animal is in motion or at rest is achieved by a number of complex reflex activities grouped under postural reactions. The general behavior of animals in the critical stage was indicative of functional disturbances of these reflexes, and a detailed study was therefore undertaken.

Tonic labyrinthine reflexes. If a blindfolded normal animal is lifted by the hind limbs, the animal dorsiflexes the head and neck bringing the head into a plane as nearly parallel with the floor as possible. Animals in the critical stage do not show the normal reaction, the head remaining ventroflexed. Further, when the normal animal is held in the air supine the head tends to turn, while cats in the critical stage show spastic head ventroflexion which usually remains unchanged no matter in what position the animal is held.

Righting reactions. The righting reactions of the cat are developed to an exceptional degree in regard to their speed and accuracy. In startling contrast to the normal is the almost complete loss of body righting in air observed in the critical stage. When such an animal is held in the supine position the limbs are usually extended and abducted and the head ventroflexed. Upon releasing the cat this position remains "frozen" throughout the fall. However, when the back of the animal contacts the surface of the pillow righting occurs immediately.

Body righting reactions acting on the head. This righting is initiated by the unequal stimulation of the two sides of the body. Cats in the critical stage when laid on their side are able to right themselves. The same reaction apparently accounts for the righting which occurs when the animal falls on its back. This is the only righting reflex studied which remains normal.

Vestibulo-ocular reflex (nystagmus). In order to obtain more specific information with regard to the functional condition of the semi-circular canals and associated reflex pathways, a study of postrotatory nystagmus was made on the series of 10 cats during the course of thiamin deficiency. The experiments were conducted with a specially constructed revolving table. The animal was placed with the head over the center of rotation. Considerable care was taken in centering the head, which was fixed securely and in a horizontal plane. An electric motor turned the table at 120 revolutions per minute. The animals were rotated for one minute. The motor was then stopped and ten seconds later the revolving table was brought to a halt. Nystagmus time and the number of excursions of the eyes were then taken. Duplicate runs on the same animal checked within a second and variation from day to day in normal controls was never greater than three seconds.

A consideration of the typical data presented in table 1 reveals the wide variation in nystagmus time in different cats. It is necessary, therefore, to study each case separately in determining the possible effects of thiamin deficiency. During the induction stage in the cat the nystagmus time tends to decrease slightly, but the change is so small as to be of doubtful significance. When the animal reaches the critical stage a striking decrease in the duration of nystagmus occurs. Also the eye movements tend to be small and erratic. In contrast to these findings are those of Church (1935) who found that nystagmus time became progressively greater during the development of deficiency in the rat up to the

TABLE 1

Postrotatory nystagmus time and characteristics in normal and thiamin deficient cats

CONDITION OR STAGE	DAYS ON DIET	NYSTAGMUS TIME	NUMBER OF EXCURSIONS	TYPE OF EXCURSION
		seconds		
Normal		36	98	Normal, large
Induction	4	37	97	Normal, large
Induction	14	32	97	Normal, large
Critical	28	16	26	Erratic, small
2 days post B_1*		19	41	Normal, large
Normal		22	60	Normal, large
Induction	4	23	65	Normal, large
Induction	14	22	60	Normal, large
Critical	31	5	4	Erratic, small
Normal		22	30	Normal, large
Induction 1	19	17	39	Normal, large
Critical	29	4	4	Erratic, small
2 days post B_1		24	40	Normal, large
Normal		36		Normal, large
Induction	16	35		Normal, large
Induction	22	34		Normal, large
Critical	30	20		Erratic, small
Critical	31	16		Erratic, small
1 day post B_1		22	26	Normal, large

* Number of days after injection of 1 mgm. of thiamin and return to normal diet.

time of the onset of disturbances of equilibrium and then decreased precipitously. These rats were on a diet in which autoclaved yeast was used as the only source of B-complex vitamins and therefore the possibility of other vitamin deficiencies occurring cannot be overlooked.

The depression of the vestibulo-ocular reflexes during the critical stage is a reversible phenomenon, for after recovering the cat by injecting thiamin, nystagmus time increases toward normal and the ocular movements are of the large normal type.

Hopping and placing reactions. These postural reactions have been studied

in the cat by Bard (1933) and shown to have a strict cortical localization in the cerebrum. These two reflexes are present in cats on diet up to and including the critical stage.

3. Light reflex. This reflex is present but pupillary constriction is less than normal. For a given light intensity, the pupils of a deficient cat in the critical stage are approximately twice the size of those of a normal animal.

4. Cerebellar function. Special attention was directed to the study of limb and body movements when the animals were walking. These observations revealed some of the typical signs associated with cerebellar lesions, namely, ataxia, dysmetria and asynergia. These three conditions result in a staggering unco-ordinated, weaving gait with the animal often lifting the limbs too high and losing its balance.

5. Cerebrocortical function. It has been mentioned that the behavior of the thiamin deficient cat indicates little impairment of cortical function. Further, the ability of the animal to follow an object with the eyes shows that sight is present as does also the avoidance of obstacles when walking about. That the animal hears is indicated by the presence of startle to loud noises and the turning of the ears to less intense sounds. The integrity of the hopping and placing reactions is also evidence of normal cortical function.

Discussion. The presence of normal flexor response and knee jerk make it highly improbable that any of the observed signs of thiamin deficiency are due to impairment of the sensory or motor end organs and associated nerve fibers Further the widespread nature of the disorders and their almost simultaneous appearance direct attention to the central nervous system as the site of these functional disturbances.

The functions of the cerebral cortex do not appear to be markedly impaired. Rather, it is in the postural mechanism that the most striking abnormalities are observed. The abnormal posture and loss of righting in air most probably result from the functional impairment of the reflexes arising chiefly in the otolithic maculae. These righting reactions are integrated in the reticular substance at the level of the red nuclei in the midbrain according to Ingram, Ranson and Harris (1934).

The decreased nystagmus time observed is indicative of a disturbance in the vestibulo-ocular reflex pathway which has synapses in the nuclei of the third, fourth and sixth nerves lying in the midbrain and pons. The ataxia, asynergia and dysmetria observed may arise from a lesion in the red nuclei of the midbrain. Ingram and Ranson (1932) have shown that destruction of the red nuclei in the cat leads to signs similar to those seen following ablation of the cerebellum.

A constant accompaniment of the critical stage is the pupillary dilatation which is at all times greater than normal and becomes maximal during and immediately after convulsions. Release of adrenalin during the seizures may be involved in the dilatation seen at this time. However, a disorder in the reflex pathway, possibly in the pretectal region of the midbrain, seems more probable since the pupil dilatation becomes progressively greater and finally maximal in the terminal stage when convulsions no longer occur.

From these considerations it is seen that the functional nervous disorders observed in the thiamin deficient cat involve reflex pathways which have important synaptic centers in the midbrain. Functional disturbances of the nuclear masses in the upper portion of the brain stem would therefore account for most of the observed signs. From his studies on beriberi rats, Church (1935) described a similar locus to account for the neurological disorders which are in many respects similar to those seen in the cat. Prickett (1934) described marked cellular damage in the region of the vestibular nuclei and cerebellum of chronic thiamin deficient rats.

It is more difficult to locate the origin of the convulsive seizures. Generally, it is assumed that these result from cortical activity. However, the short duration of the convulsions in the thiamin deficient cat, lasting for only a few seconds in most cases, militates against cortical origin. Rosenblueth and Cannon (1942) in a study of the character of cortical discharge found activity lasting for a minute or more. Possibly the seizures observed in the critical stage animals may have a subcortical origin, perhaps in the midbrain itself.

Since the onset of nervous disorders is very sudden it appears that the deficiency reaches a critical level beyond which normal function is no longer possible. The simultaneous appearance of the signs of the critical stage is suggestive of a localized functional lesion. The anorexia and vomiting which occur much earlier most likely involve a different mechanism, possibly a disturbance of tone or absorptive capacity of the gastro-intestinal tract.

The onset of the critical period is one of dysfunction not entirely depressive in character. The hypertonia, restlessness and seizures are more indicative of hyperexcitability or release phenomena. As the animal progresses into the late critical stage, this excitability gives way rapidly to general depression and finally the irreversible changes associated with the terminal stage.

SUMMARY AND CONCLUSIONS

1. A dietary regime, having as a feature the use of crystalline vitamin supplements given both orally and intraperitoneally, has been shown to meet the requirements for the production of specific thiamin deficiency. Thus, the diet was shown to maintain the animal in normal health if thiamin was added. Further the disorders arising while the animals were on diet, namely, anorexia in the early period and postural disturbances and convulsions in the critical stage, were specifically alleviated within a period of a day after the injection of thiamin. Injection of other B complex vitamins had no effect.

2. The development of thiamin deficiency in the cat may be divided into three stages: 1, induction stage, characterized by the development of anorexia; 2, critical stage, characterized by the sudden appearance of many neurological disorders, particularly of the postural mechanisms, and usually short tonic convulsive seizures. The animal becomes progressively weaker and passes into 3, the terminal stage where the animal is prostrate and cannot be recovered; death follows within a day or two.

A detailed study of the reflex activities of cats in the critical stage revealed impairment of 1, labyrinthine righting reactions as shown by the abnormal head

position and loss of righting in air; 2, vestibulo-ocular reflex as indicated by the decreased nystagmus time; 3, pupillary light reflex as revealed by the increase in pupil size. Dysfunction of the cerebellum was suggested by the presence of asynergia, ataxia and dysmetria.

A consideration of the reflex pathways involved in the reactions which showed impairment revealed that all of them have synaptic centers in the midbrain. This is proposed as the probable locus of the functional lesion giving rise to the observed nervous disorders.

Acknowledgments. The author wishes to express his appreciation to Dr. D. C. Smith for his interest and helpful direction of this work, and to Dr. J. E. P. Toman for suggestions in the preparation of the neurological studies.

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ESTIMATION OF PLATELET FRAGILITY[1]

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Received for publication March 2, 1944

A strain of swine with a defective blood clotting mechanism has been described in earlier reports (1, 2, 3). It was reported, along with other observations, that when studied by the procedure of Howell and Cekada (4) the platelets from the defective animals disintegrated more slowly than did those from normal animals. The rate of disintegration, by this procedure, is measured by microscopical examination of blood smears taken from samples of coagulating blood. The intermediate grouping and agglutination during the stage of platelet break-down makes the end point indefinite and the method is highly subjective.

The purpose of this investigation was to devise an objective method of measuring the fragility of blood platelets, and to compare the fragility of the platelets in bleeder and in normal swine blood.

EXPERIMENTAL. The method adopted for the measurement of platelet fragility is based on the fact that platelets, in the same manner as red cells, are ruptured by hypotonic solutions. It is assumed for the present that when platelets disintegrate they release thromboplastin which accelerates the rate of coagulation in recalcified plasma. The rate of coagulation then is an indirect measure of the extent of platelet disintegration, or platelet fragility. The procedure for estimating platelet fragility is a modification of Sanford's method (5) of estimating erythrocyte fragility:

1. Arrange in order of concentration a series of 10 glass test tubes (75 mm. x 10 mm.) which contain sodium chloride solutions of varying concentrations. Each tube contains 0.75 ml. and the concentrations of the salt, in percentages, are: 0.0, 0.3, 0.5, 0.7, 0.8, 0.9, 1.2, 1.5, 2.0 and 2.5.

2. Draw 8 ml. of blood, by venous puncture, directly into a cold paraffined centrifuge tube containing 2 ml. of 3.8 per cent trisodium citrate ($Na_3C_6H_5O_7 \cdot 5.5H_2O$). Keep the tube in an ice bath until centrifuged.

3. Centrifuge the blood lightly, just long enough to remove the erythrocytes from the cloudy, platelet-laden plasma (1000 R.P.M. for 8 minutes in an angle centrifuge with an outside diameter of 25 cm.). Centrifugation should be completed in less than 30 minutes after the blood is collected.

4. Add 0.25 ml. of plasma and 0.1 ml. of 2.5 per cent $CaCl_2 \cdot 2H_2O$ to each of the 10 tubes described in no. 1.

5. Thoroughly mix the contents of the tubes by shaking. Tilt the tubes at 30 second intervals in order to ascertain the coagulation time. The coagulation time includes the period between addition of the calcium ion and completion of the coagulation process.

[1] Aided by a grant from the John and Mary R. Markle Foundation. Journal Series no. 938.

If more convenient, the coagulation step can be carried out at room temperature since, according to Cheney (6), a variation between 23° and 26°C. has little

TABLE 1

Relation of cloudy plasma coagulation time to salt concentration

ANIMAL NO.	% NaCl USED AS DILUENT										TEMPERATURE WHEN CENTRIFUGED °C.
	0.0	0.3	0.5	0.7	0.8	0.9	1.2	1.5	2.0	2.5	
	Coagulation time in minutes										
80 B*	2.5	4.0	7.0	9.0	9.0	9.5	10.5	17.0	32.0	65.0	25
1 B	3.0	4.0	7.5	10.5	9.5	9.5	13.0	18.5	34.0	85.0	
126 B	2.5	3.5	6.0	8.0	8.0	8.5	12.0	16.0	24.0	63.0	
108 B	2.5	4.0	7.0	7.5	7.0	8.5	9.5	14.0	28.0	71.0	
127 B	2.5	3.5	6.0	7.5	6.5	8.5	9.5	14.5	30.0	57.0	
Average....	2.6	3.8	6.7	8.5	8.0	8.9	10.9	16.0	29.6	68.2	
34 N†	2.5	2.5	4.0	5.0	5.0	6.0	6.5	8.0	14.0	18.0	25
70 N	3.0	4.5	4.5	4.5	5.0	5.5	6.5	8.0	16.0	40.0	
85 N	2.0	2.5	4.0	4.5	4.5	5.0	5.0	8.0	12.0	22.0	
84 N	2.5	2.5	4.0	4.0	5.0	5.5	5.5	8.0	11.0	19.0	
91 N	2.5	3.0	4.0	4.5	4.5	5.0	6.0	8.5	12.0	20.0	
Average....	2.5	3.0	4.1	4.5	4.8	5.4	5.9	8.1	13.0	23.8	
30M B	4.0	5.0	11.0	12.0	14.0	14.5	19.5	30.5	49.0	104.0	0
35 B	4.5	6.0	7.5	9.5	14.5	18.5	21.5	29.5	34.0	61.0	
36 B	2.5	3.0	5.0	9.0	9.5	11.5	14.5	16.5	33.0	65.0	
30F B	3.0	4.5	4.5	7.0	13.5	17.5	18.5	28.0	43.0	92.0	
3 B	4.0	5.0	11.0	16.5	18.0	19.5	17.5	23.5	55.0	96.0	
Average....	3.6	4.7	7.8	10.8	13.9	16.3	18.3	25.6	42.8	83.6	
2 N	3.5	4.5	6.5	7.0	7.5	9.0	10.5	16.0	29.0	40.0	0
1 N	4.0	5.5	6.5	7.5	8.5	9.5	12.5	16.0	21.0	26.0	
3 N	3.5	4.5	5.0	5.5	6.0	7.0	7.5	12.0	21.0	37.0	
37 N	4.0	5.0	7.0	7.5	7.5	8.0	8.5	15.5	19.0	24.5	
8 N	3.5	4.5	5.5	7.5	8.5	9.0	11.0	12.5	24.0	34.0	
Average....	3.7	4.8	6.1	7.0	7.6	8.5	10.0	14.4	22.8	32.3	
Average of 10 bleeders....	3.1	4.3	7.3	9.7	11.0	12.6	14.6	20.8	36.2	75.9	Combined
Average of 10 normals....	3.1	3.9	5.1	5.8	6.2	7.0	7.9	11.3	17.9	28.1	Combined

* Bleeder.
† Normal.

effect on the plasma coagulation time. During this investigation, however, the tubes were kept in a constant temperature water bath at 25°C. In the first

series of trials the blood was centrifuged at room temperature, but in later trials the temperature was reduced to 0°C. This change was made in order to delay platelet breakdown during the period preceding recalcification.

When the plasma is diluted with hypotonic salt solution some platelets are necessarily subjected momentarily to a lower tonicity than that of the final dilution. Whether the platelets disintegrate during or after dilution, the coagulation time is an index to the extent of platelet break-down. The experimental conditions in the normal and bleeder plasmas are the same, and any differences in the results are presumably due to differences in the platelets.

The data on plasma coagulation time are shown in detail in table 1 and shown graphically in figure 1.

TABLE 2

The interrelation of coagulation time, platelet quality, platelet quantity and salt concentration

PLATELET CONCENTRATION	KIND OF ANIMAL	NO. OF ANIMALS AVERAGED	% NaCl USED AS DILUENT									
			0.0	0.3	0.5	0.7	0.8	0.9	1.2	1.5	2.0	2.5
			Coagulation time in minutes									
Whole blood	Normal	5	4.5	5.4	6.0	7.5	7.9	8.4	9.9	13.8	20.9	32.6
	Bleeder	5	3.6	4.8	8.0	9.6	11.2	12.2	16.2	22.5	39.6	132.3
Cloudy plasma (platelet rich)	Normal	10	3.1	3.9	5.1	5.8	6.2	7.0	7.9	11.3	17.9	28.1
	Bleeder	10	3.1	4.3	7.3	9.7	11.0	12.6	14.6	20.8	36.2	75.9
Clear plasma (platelet poor)	Normal	5	8.3	8.4	8.6	9.0	10.0	10.1	15.0	20.7	28.6	930.8
	Bleeder	5	14.9	15.8	20.5	21.1	21.8	25.4	39.3	68.0	218.0	∞
Filtered plasma (platelet free)	Normal	5	17.1	17.1	18.2	19.4	19.0	26.1	136.8	166.5	∞	∞
	Bleeder	5	74.8	73.9	75.3	75.8	85.3	95.2	838.1	∞	∞	∞

∞ = more than 3200 minutes.

According to the conventional interpretation the data indicate that if the solution is sufficiently hypotonic to cause complete disintegration, bleeder platelets liberate as much thromboplastin as do normal platelets. However, as the salt concentration approaches the physiological level, the bleeder platelets disintegrate less rapidly and, therefore, liberate less thromboplastin than do normal platelets. Apparently bleeder platelets in 0.5 per cent sodium chloride disintegrate at about the same rate as do normal platelets in 0.9 per cent sodium chloride solution. This comparison supports the hypothesis that bleeder platelets are abnormally stable.

In an effort to determine whether or not the accelerated rate of coagulation in hypotonic solutions is due to platelet disintegration the technique was repeated on plasmas containing different concentrations of platelets. Clear, platelet-poor plasma was prepared by centrifuging the blood at 4000 R.P.M. in an angle centrifuge for one hour at 0°C. Platelet-free plasma was prepared by filtering the

clear plasma through a sintered glass filter (diameter of largest pore = 7.6μ). Whole blood was included in the study to observe the combined effect of erythrocytes and platelets. The coagulation time of these preparations, with varying concentration of sodium chloride, was determined by the methods previously described and the data are summarized in table 2. The relation between the coagulation time of bleeder and normal platelet-free plasma is presented graphically in figure 2.

Discussion. The data in table 2 show that the number of platelets, the characteristics of the platelets, and the concentration of salt are all factors in the coagulation time of plasma. The addition of water to cloudy plasma decreased the coagulation time of bleeder and normal plasma to the same low level (fig. 1),

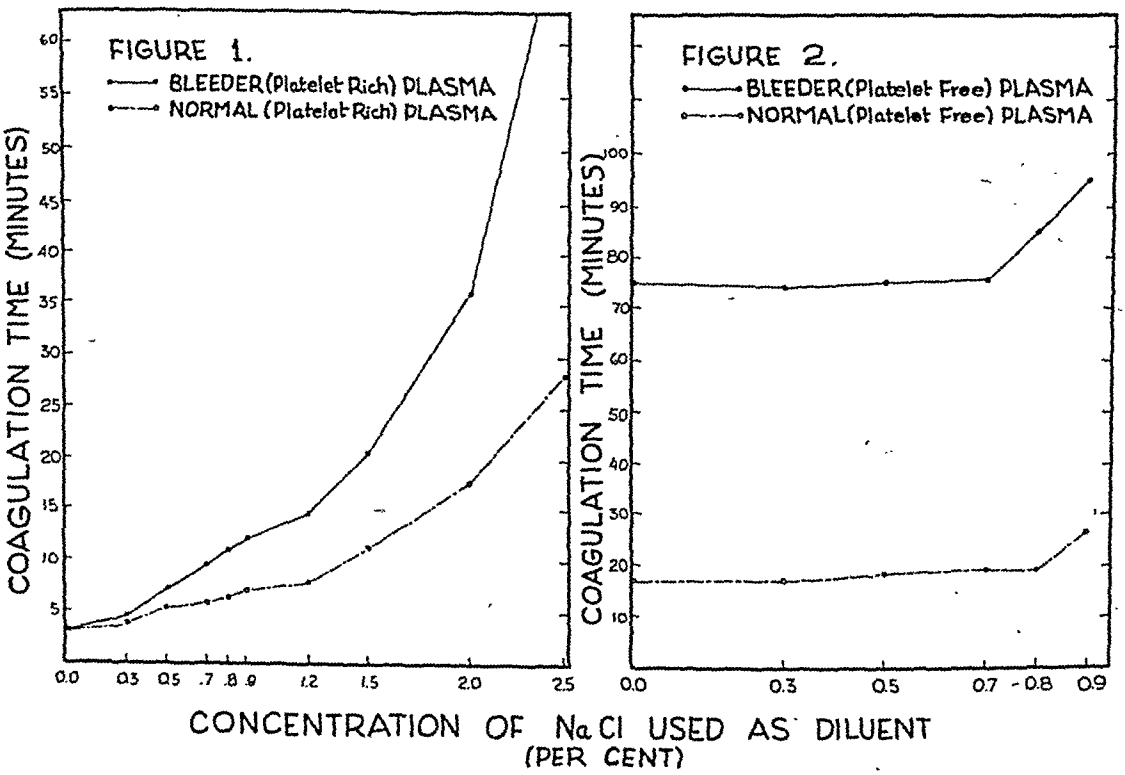

Fig. 1. The effect of salt concentration on the coagulation time of platelet-rich plasma.
Fig. 2. The effect of salt concentration on the coagulation time of platelet-free plasma.

but the addition of water to platelet-free plasma had a negligible effect on the coagulation time (fig. 2). When physiological saline, or any of the more dilute solutions was added, the coagulation time of bleeder platelet-free plasma was consistently about four times longer than that of normal platelet-free plasma. The more labile normal platelets liberated thromboplastin before they were centrifuged and filtered out and the resulting plasma coagulated in a comparatively short time. The relatively stable bleeder platelets, however, could be removed before they appreciably disintegrated and the resulting thromboplastin-deficient plasma coagulated slowly. The differences in coagulation time of platelet-free plasma from bleeder and from normal animals cannot be eliminated by decreasing the tonicity of the solution (fig. 2). Since the coagulation time of platelet-free plasma cannot be reduced to a normal level by dilution, this reduced

time in cloudy plasma is probably explained by platelet activation. Likewise, as would be expected from these observations, when the tonicity of clear, platelet-poor plasma was reduced (table 2), there was no decided reduction in coagulation time, though the fall was more evident in the bleeder preparation. In whole blood, however, lowered tonicity decreased the coagulation time far below the normal level in both bleeder and normal samples (table 2). These data indicate that platelet stability can be reliably estimated by the technique described.

Howell and Cekada (4) did much to popularize the idea that hemophilia platelets are abnormally stable and our earlier work (1) followed their procedure which is based on microscopical examination of platelets in a sample of coagulating blood. One could assume that the coagulation changes hasten platelet break-down rather than the more popular hypothesis that the platelet break-down hastens rapid coagulation. The possibility is not excluded that a third factor initiates simultaneously blood coagulation and platelet break-down and the determination of platelet stability in a sample of coagulating blood may measure something other than an inherent platelet defect. However, in the method used in this investigation the platelet break-down is initiated by hypotonic solution before recalcification and before the coagulation process is under way. If the solution is sufficiently hypotonic to disrupt all the platelets, the platelet disintegration and plasma coagulation processes are separated. The most plausible explanation then of the data in tables 1 and 2 is that rapid coagulation is the result of the platelet break-down. However, this explanation does not exclude the possibility that coagulation may have some effect on intact platelets. Since a decrease in tonicity reduced coagulation time of both bleeder and normal platelet-rich plasma to the same low level, it is assumed that the defect in bleeder blood has some relation to abnormally stable platelets.

SUMMARY

1. The coagulation time of platelet-containing plasma is sharply reduced on dilution with hypotonic salt solution.

2. Bleeder plasma coagulates at the same rate as normal plasma at the lower salt concentrations.

3. It is assumed as a tentative explanation that bleeder platelets are more resistant to hypotonic salt solution than are normal platelets. When they do disintegrate they may release as much thromboplastin as do normal platelets.

4. The coagulation time of cloudy plasma, when made hypotonic, is an index of platelet fragility.

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THE EFFECT OF CAFFEINE UPON GASTRIC SECRETION IN THE DOG, CAT AND MAN

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Received for publication March 6, 1944

We have recently confirmed the work of Judd (1) on the experimental production of acute and subacute gastric ulcers in cats by the intramuscular implantation of caffeine in a beeswax-mineral oil mixture (2). It is to be suspected that the constant elaboration of a highly acid gastric juice might be one of the factors involved in the pathogenesis of such ulcers. However, there is no uniformity of opinion among investigators as to whether caffeine does or does not stimulate gastric secretion.

Caffeine has been shown consistently to have no effect upon gastric secretion in Pavlov and Heidenhain pouch dogs (1, 3, 4, 5, 6, 7, 8). Actual investigation of the influence of caffeine upon gastric secretion in the cat has not been made, but an excito-secretory effect has been suspected (1, 9, 10). Considerable disagreement is found in the literature concerning the effect of caffeine in man. Various authors (6, 11, 12, 13, 14) conclude that caffeine does not stimulate gastric secretion in man; whereas others (15, 16, 17, 18) have observed an increased output of acid gastric juice after caffeine administered orally or parenterally. The experimental design and technique used in some of the investigations on human subjects was poor in the light of current knowledge.

The purpose of the present investigation was to determine whether caffeine stimulates gastric secretion in man, cat and the dog, and whether the apparent species difference does exist.

METHODS. *Dogs.* Four dogs with Pavlov pouches of the stomach and two dogs with gastrostomies were used in these studies. In all experiments, before caffeine was administered, the animals were allowed to become basal, i.e., secreting no free acid. The dose of caffeine used was 250 mgm. as the sodium benzoate. The caffeine was usually given subcutaneously, but in a few experiments the intramuscular and intravenous routes were used. Following the injection of caffeine, the gastric juice was collected for a two-hour period. In order to demonstrate that these gastric pouches were capable of responding to a known stimulus, histamine was injected subcutaneously two hours after the administration of caffeine and the gastric juice collected for another one-hour period. The histamine used was the dihydrochloride; the dose varied from 0.2 to 0.4 mgm., but was kept constant for any given animal.

Cats. Acute experiments on cats under chloroform anesthesia were conducted using a modification of the technique devised by Lim (19) which consists of ligation of the cardio-esophageal junction, excluding the vagi by previous dissection, and cannulation of the stomach with a perforated rubber tube inserted through the pylorus. With cats prepared in this manner, the effect of caffeine on gastric

secretion was studied. Caffeine was administered intravenously in doses of 65 to 125 mgm. as an aqueous solution of caffeine with sodium benzoate. As the drops of gastric juice fell from the cannula, they were recorded on a slowly moving kymograph. From this record the rate of flow (drops per minute), latent period and duration of response were calculated.

In another group of experiments, 100 mgm. of caffeine in 20 cc. of distilled water, neutralized to pH 6, was introduced through the pyloric cannula and allowed to remain in contact with the gastric mucosa for 30 minutes. After this interval the gastric contents were drained and the stomach rinsed twice with distilled water. The gastric secretion was collected for a period of 90 to 120 minutes, washing the stomach every 30 minutes.

Human subjects. Fractional gastric analyses were conducted on human subjects using a "block" technique to permit exposure of the gastric mucosa to the test meal for a period of 30 minutes. All tests were performed after a fast of 10 to 12 hours. To obtain the "basal" level of secretion, the stomach was emptied completely of its contents every 10 minutes for a control period of one-half hour. By means of a large syringe, 200 cc. water (temp. 37–40°C.) containing 250 mgm. sodium benzoate was introduced into the stomach via the Rehfuss tube, serving as a control for the volume and chemical influence of sodium benzoate in the caffeine test meal. For the next two 10-minute intervals, 10 cc. samples of gastric contents were removed for analysis. The purpose in removing these samples was to detect the first rise in acid concentration and thus the latent period. Thirty minutes after the control test meal was introduced and every ten minutes thereafter until the secretory response had subsided and the "basal" level once again was obtained, the stomach was emptied completely of its contents. As pointed out by Bloomfield and Keefer (20) the complete emptying of the stomach is made more certain by having the subject lean backward, forward, and from side to side as the stomach is aspirated. Having thus obtained the "basal" level of secretion and the secretory response to a control test meal, an equal volume of water containing 250 mgm. caffeine as the sodium benzoate was introduced via the stomach tube and the "block" procedure again conducted as outlined above.

The total output of acid was calculated on the basis of the titration values for the two 10 cc. samples plus the complete recovery every 10 minutes thereafter for a period of 1 hour. Thus, since the volume of gastric contents aspirated depends upon not only the secretion but the amount lost through the pylorus, the total output of acid would be greater than that represented in the data.

Another group of experiments were made on human subjects to study the gastric secretory effect of caffeine administered intramuscularly. After a "basal" control period of 30 minutes, 250 mgm. caffeine were injected intramuscularly and the total gastric contents aspirated every 10 minutes for at least one and one-half hours.

All samples of gastric juice in these experiments were titrated against 0.0278 N NaOH (1 cc. equals 1 mgm. HCl) for free and total acidity, using Töpfer's reagent (pH 3.5) and phenolphthalein (pH 8.5) respectively. In all cases the responses are expressed as the output of acid in milligrams of HCl.

RESULTS. *Dogs.* The results of 32 experiments on 6 dogs are summarized in the accompanying table 1. In no instance did the parenteral administration of caffeine stimulate gastric secretion in the dog, but histamine gave rise to its usual

TABLE 1

The gastric secretory response to caffeine and histamine in dogs

DOGS	DOG NO.	NO. OF EXPTS.	AVE. POST-CAFFEINE RESPONSE (MGM. HCl/2 HRS.)		AVE. POST-HISTAMINE RESPONSE (MGM. HCl/1 HR.)	
			Free acid	Total acid	Free acid	Total acid
Pavlov pouch dogs	P-1	8	0.0	2.7	57	75
	P-2	6	0.0	7.8	53	66
	P-3	6	0.0	5.2	76	97
	P-4	6	0.0	2.3	31	46
Gastrostomy dogs	G-1	3	0.0	5.0	23	32
	G-2	3	0.0	11.0	54	98
Ave.....	6	32	0.0	5.8	49	69

TABLE 2

The gastric secretory response to intravenous caffeine in cats*

CAT NO. VAGI INTACT	PRE-CAFFEINE RATE	LATENT PERIOD	MAX. POST-CAFFEINE RATE	DURATION OF RESPONSE	VOLUME AND MGM. FREE HYDROCHLORIC ACID			
					Pre-caffeine (10 min.)		Post-caffeine (60 min.)	
					cc.	mgm. HCl	cc.	mgm. HCl
	drops/min.	*min.*	*drops/min.*	*min.*				
1	0.33	20	5.00	30	0.4	0.0	8.3	41.8
2	0.08	13	1.84	76	0.6	0.4	4.5	6.6
3	0.38	13	1.23	34	0.3	0.2	2.7	11.6
4	0.64	20	1.17	48	0.2	0.6	2.9	6.7
5	0.34	12	1.03	39	0.1	0.0	2.7	11.0
6	0.23	7	4.00	50	0.3	0.0	8.3	20.6
7	0.11	12	1.89	30	0.2	0.1	2.2	2.0
8	0.12	10	2.58	61	0.1	0.0	5.2	14.2
9	0.41	10	1.36	39	0.2	0.0	5.2	22.1
10	0.23	6	3.86	47	0.1	0.1	5.9	19.4
11	0.38	9	1.50	35	0.2	0.0	4.1	8.8
12	0.06	12	3.00	56	0.3	0.0	7.4	29.4
13	0.40	17	2.60	40	0.1	0.0	2.3	13.5
14	0.20	10	2.50	50	0.2	0.0	3.3	15.7
Ave. ..	0.26	12	2.4	45	0.23	0.1	4.7	16.0

* Dose: 125 mgm. caffeine as the sodium benzoate. Cat 13 received 65 mgm.

excitosecretory response. Lavage of the stomach with caffeine in Pavlov and total pouch dogs does not provoke a stimulation of gastric secretion.

Cat. The data in table 2 show that the intravenous injection of caffeine in anesthetized cats provokes a marked stimulation of gastric secretion. The usual

sequence of events following the intravenous administration of caffeine include: marked mydriasis for 15 to 30 minutes, hyperpnea, and, after an average latent period of 12 minutes, the increased flow of acid gastric juice was manifest, lasting on the average for a period of 45 minutes.

TABLE 3

Average gastric secretory response to lavage with caffeine in cats

	TIME	VOLUME	FREE ACID	TOTAL ACID
	minutes	*cc.*	*mgm. HCl*	*mgm. HCl*
Control secretion	30	0.4	0.0	1.4
Caffeine soln. applied	30	20.0	0.0	6.0
Caffeine soln. returned		20.7	1.8	7.3
Post-application secretion	30	1.8	6.2	7.6
Post-application secretion	30	1.0	2.7	3.6
Post-application secretion	30	0.7	1.1	1.9

TABLE 4

The gastric secretory response to oral administration of caffeine in human subjects

SUBJECT	CONTROL: 200 CC. WATER + 250 MGM. SODIUM BENZOATE			EXPERIMENTAL TEST: 200 CC. WATER + 250 MGM. CAFFEINE—ORAL					PER CENT OF STIMULATION (FREE ACID)
	Total juice secreted in 1 hour:			Latent period	Duration of response	Total juice secreted in 1 hour:			
	Volume	Free acid	Total acid			Volume	Free acid	Total acid	
	cc.	*mgm. HCl*	*mgm. HCl*	*minutes*	*minutes*	*cc.*	*mgm. HCl*	*mgm. HCl*	
J. R.	51	15	27	15	30	73	98	113	550
J. R.	51	7	16	15	50	55	55	63	685
E. P.	67	99	115	15	40	95	195	218	97
J. I.	180	228	282	15	60+	199	363	435	59
J. W.	42	12	23	15	50	48	36	48	200
J. A.	139	9	33	15	50	77	28	49	211
P. H.	46	17	29	15	60	94	181	218	964
J. J.	58	67	81	15	60+	142	337	392	403
F. J.	81	190	212	15	60+	123	393	441	107
G. H.	64	60	82	25	60	93	152	186	153
M. S.	52	47	64	25	60	61	107	144	128
Ave.	75	69	88	17	53	93	177	210	324

In 15 experiments on 9 cats, lavage of the stomach for 30 minutes with caffeine resulted in a stimulation of gastric secretion as measured by the volume, free acid and total acid response (table 3). The same volume of water (20 cc.) has been shown to have no effect in a series of control experiments.

Man. In table 4 are presented the individual variations in the gastric secretory response to the administration of caffeine via the stomach tube in human subjects.

In general three types of secretory curves are obtained by the fractional gastric analysis with a caffeine test meal. Approximately 85 per cent of human subjects give an abrupt curve which returns to its basal level within 50 to 70 minutes; 10 per cent describe a low flat curve (subjects J. W. and J. A.) which subsides within 60 to 90 minutes; and about 5 per cent show a prolonged secretory response at a high plateau level (subjects J. I., J. J. and F. J.). In a comparison between the response to the control (200 cc. water with 250 mgm. sodium benzoate) and to the caffeine test meals (table 4) it appears that the average volume of secretion is not much greater after caffeine (93 cc. compared to 75 cc.). However, in using a "block" technique it must be remembered that only a sample is withdrawn at the first two intervals and then the stomach is emptied completely of its contents at the third interval. This results in the recovery of a relatively large volume at this interval in both test meals, although the acid value is but slightly elevated above the basal level in the control studies. The data on the caffeine test meal show that the stimulation of parietal cell secretion is manifest after a latent period of 15 to 25 minutes; i.e., the free acid concentration began to rise during the period between the 10 and 20 minute interval aspirations. The average free acid response is over two and one-half times greater for the caffeine test meal than that for the control in the same period of time; actually the total output is even greater because the response from caffeine is much more prolonged in all individuals than the response to the control tests.

In figure 1 the graph represents the composite gastric secretory response to a caffeine test meal in comparison with the effect of a control test meal in 10 human subjects. In analyzing the curves for acidity, the initial drop after the control test solution and to a less extent after the caffeine test meal is attributed to dilution by the test solutions inasmuch as only a sample of gastric contents is aspirated at these intervals. The abrupt rise then represents the recovery of gastric contents accumulating over the 30 minute period in which the mucosa is exposed to the stimulating agent. In this composite response the actual peak of secretion resulting from the caffeine stimulation occurs 40 to 50 minutes after the test solution was introduced. In the block form of graphic representation can be seen the average maximum secretory response in volume, free acid and total acid per 10 minutes in comparison with the average pre-caffeine control levels for a 10 minute period.

The results of the gastric secretory response to caffeine given intramuscularly in human subjects are summarized in table 5. This route of administration was used in man to demonstrate that caffeine stimulation is *not solely* dependent on the local effect of the drug on the mucosa. The tabulated data show the individual variations in latent period, duration of stimulation and the total output of gastric juice for a period of 90 minutes after the injection of caffeine. The same subjects who gave a prolonged response to the caffeine test meal via the stomach tube gave a similar response to the intramuscular caffeine but at a somewhat lower plateau level.

In figure 2 the composite secretory response to the intramuscular administration of caffeine in 14 human subjects is presented in the form of a graph. The

composite curves are misleading in so far as the latent period is concerned. The majority of subjects do not show evidence of stimulation for 50 to 60 minutes after the injection, but a few do after 20 to 30 minutes. Hence the early rise in the composite curves. The block graph shows the average maximum secretory response per 10 minutes after intramuscular caffeine in comparison with the average basal levels for the 10 minute interval preceding injection.

Discussion. The dog has been the most frequent choice of experimental animal for studies on the mechanisms of gastric secretion, on the production of

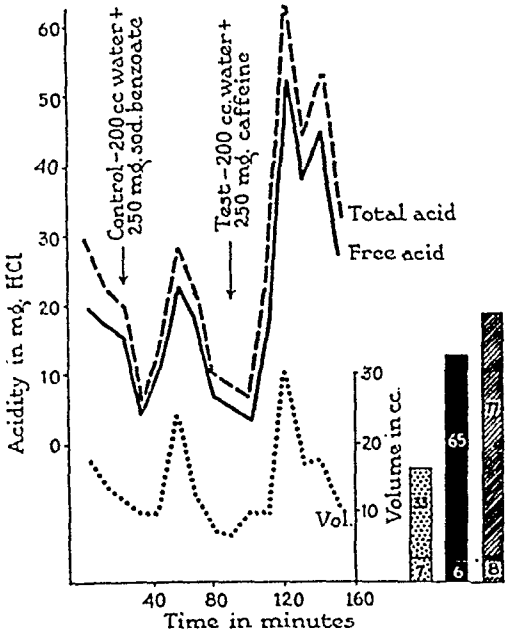

Fig. 1

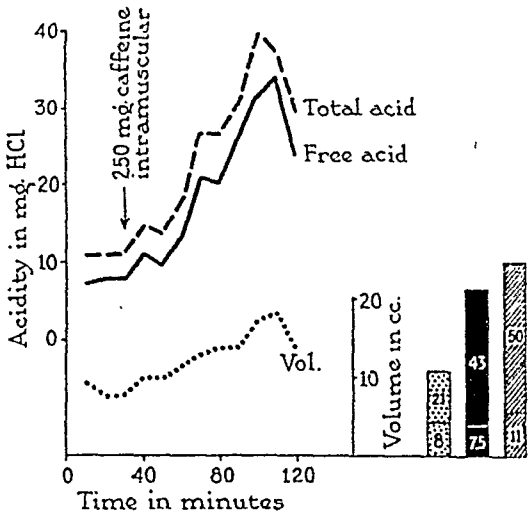

Fig. 2

Fig. 1. Composite curves showing the average total gastric secretory response of 10 human subjects to a control test meal (200 cc. water with 250 mgm. sodium benzoate) and a caffeine test meal (200 cc. water with 250 mgm. caffeine as a soluble sodium benzoate). Both free and total acid are expressed as milligrams of HCl. The block graph shows a comparison between the average pre-caffeine levels of volume, free acid and total acid per 10 minutes and the average maximum secretory response for 10 minutes.

Fig. 2 Composite curves showing the average total gastric secretory response of 10 human subjects to the intramuscular administration of 250 mgm. caffeine as the sodium benzoate. The block graph shows a comparison between the average pre-caffeine levels of volume, free acid and total acid per 10 minutes and the average maximum secretory response for 10 minutes.

gastroduodenal ulcers, and on the effectiveness of various therapeutic procedures on experimentally induced peptic ulcers. Therefore, the observation that caffeine does not stimulate gastric secretion in the dog but does in man and the cat is very significant and has far-reaching implications. That such a species difference does exist must constantly be borne in mind in applying observations made on the dog to various physiological phenomena in man. Do man and the cat possess a mechanism of gastric secretion absent in the dog? Or, is there an effective mechanism antagonistic to the caffeine excitosecretory effect in the dog, and absent in man and the cat?

That earlier investigators failed to show conclusively that caffeine has a definite excitosecretory effect upon the stomach of man can in part be attributed to faulty technique, such as insufficient exposure of the gastric mucosa to caffeine (oral) or failure to wait long enough for intramuscular caffeine to produce its effect. We have observed the peak of gastric secretion to occur 40 to 50 minutes after the ingestion of caffeine and 70 minutes after intramuscular administration. To further clarify the confusion in the literature regarding the effect of caffeine and caffeine-containing beverages upon gastric secretion, the following may be said: Stimulation of gastric secretion by caffeine and coffee (or de-caffeinated prepara-

TABLE 5

The gastric secretory response to the intramuscular administration of caffeine in human subjects

SUBJECT	CONTROL PERIOD: BASAL SECRETION				EXPERIMENTAL TEST: 250 MGM. CAFFEINE—INTRAMUSCULAR					
	Basal volume	Secretion for ½ hr.		Pre-injection conc.†	Latent period	Maximum post-injection conc.†	Duration of response	Total volume	Secretion for 1½ hrs.	
		Free acid	Total acid						Free acid	Total acid
	cc.	*mgm. HCl*	*mgm. HCl*	*mgm./cc.*	*min.*	*mgm./cc.*	*min.*	*cc.*	*mgm. HCl*	*mgm. HCl*
J. R.	10	2	6	0.16	60	2.15	80	83	105	137
J. R.	18	6	13	0.29	50	1.57	80	83	77	121
M. G.	21	22	30	0.94	40	3.72	70+	170	438	525
J. R.	25	11	21	0.00	30	1.86	70	136	171	224
J. I.	50	99	123	1.73	60	2.86	60+	194	438	525
E. P.	23	66	76	2.17	60	4.07	50	76	253	296
J. R.	24	2	10	0.33	60	1.30	50	104	62	101
J. W.	29	7	24	0.20	50	1.28	40	104	80	135
F. J.	14	45	50	3.03	50	3.33	70+	115	334	383
J. A.	37	2	19	0.00	50	0.80	40	77	20	51
J. J.	12	25	30	1.30	40	2.19	70+	181	307	396
G. H.	49	4	22	0.14	30	1.73	70	181	132	211
J. N.	30	20	35	0.32	30	2.85	30	106	147	199
R. N.*	14	0	15	0.00	20	1.34	40	143	173	216
Ave.	25	22	34	0.77	45	2.22	63	125	196	251

* Secretion aspirated for only 70 minutes in this case.

† Concentration of free acid as milligram HCl per cubic centimeter per 10 minute period.

tions) are not one and the same. Thus, although a so-called de-caffeinated coffee preparation was found to provoke a flow of acid gastric juice only slightly different from that obtained from crude or roasted coffee (13), this is not evidence that caffeine does not stimulate gastric secretion in man. Nor can one conclude that the roasting process does not produce a gastric secretagogue from the crude green coffee bean. Coffee made from roasted beans may stimulate gastric secretion due to the effect of one or all of the following: *1*, its caffeine content; *2*, a natural secretagogue present in the green coffee bean; *3*, a product of the roasting process, or *4*, irritant volatile oils.

Inasmuch as caffeine, administered parenterally or by direct lavage of the stomach, does stimulate gastric secretion in the cat, the constant elaboration of a highly acid gastric juice might be one of the factors involved in the experimental development of caffeine ulcers in cats. Preliminary experiments on human subjects have also shown that caffeine stimulates the output of pepsin as well as hydrochloric acid. Thus, the excessive use of caffeine-containing beverages may contribute to the pathogenesis of human peptic ulcer in the susceptible individual, or perpetuate an ulcer already existing. This is particularly suggested by the prolonged secretory response to caffeine obtained in a certain group of subjects who fall in the descriptive category of: "conscientious, hard-working individuals who in time of stress are outwardly composed but inwardly tense."

CONCLUSIONS

1. We have confirmed the observation of the earlier investigators that caffeine does not stimulate gastric secretion in the dog.
2. Caffeine, administered by the intravenous route or by lavage of the stomach, provokes a copious flow of acid gastric juice in the cat. The significance of this species difference is pointed out.
3. Caffeine, administered intramuscularly or by the oral route, stimulates gastric secretion in man.

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THE INFLUENCE OF AGENTS AFFECTING THE AUTONOMIC NERVOUS SYSTEM ON THE MOTILITY OF THE SMALL INTESTINE[1]

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Received for publication March 9, 1944

Considerably more work has been reported on the influence of the autonomic nervous system on the intact stomach than on the intact small intestine. It is well known that the motility of the stomach is appreciably affected by the sympathetic and parasympathetic divisions of the autonomic nervous system. It is thought also that these same nerves markedly influence the motility of the small intestine. But little quantitative data as far as the authors are aware have been published on the effect of the autonomic nervous system on the propulsive motility of the intact small intestine in large mammals, such as the dog.

Our interest became aroused in this problem, because we reported recently that in the dog anoxic anoxia had no effect on the motility of the small intestine (1). This type of anoxia, however, does decrease gastric motility. Since anoxic anoxia presumably exerts its principal action by stimulation of the sympathetic division of the autonomic nervous system, it was thought worthwhile to study the action of certain agents, which are known to affect the autonomic nervous system, on the motility of the small intestine. The experiments were not designed to study the mode of action of anoxia by drugs, but rather to study quantitatively the effect of certain autonomic agents on the motility of the small intestine in the dog.

METHODS. The animals chosen for this study were unanesthetized dogs. Macht's technique essentially was employed. Matched pairs of animals were used; one to serve as a control and the other as an experimental animal. The animals had eaten no food 24 hours previous to the experiment. Approximately 50 cc. of a mixture of 10 per cent powdered charcoal suspension in 10 per cent gum acacia in water was given by stomach tube.

The effect of the following agents, all of which are known to influence the autonomic nervous system, was studied on intestinal motility: ergotamine, prostigmine, atropine, ephedrine and cocaine—in doses respectively of: $\frac{1}{10}$ mgm., $\frac{1}{2}$ mgm. 1 mgm., 25 mgm. and 10 mgm., per kilo body weight. These drugs were given intramuscularly three minutes after intubation and, of course, only one was administered to an animal. In lieu of drugs the control animals were given an intramuscular injection of normal saline solution.

Fifteen minutes after intubation, when ergotamine and prostigmine were the agents studied, the animal was sacrificed by allowing it to breathe a fatal concentration of ether. When the other three agents were studied, namely, atro-

[1]Aided by a grant of the Ella Sachs Plotz Foundation.

pine, ephedrine and cocaine, the animals were sacrificed at the end of 30 minutes. It is known, of course, that ether abolishes intestinal motility as soon as it is administered. The small intestine was removed, slit open and the distance the charcoal mixture had traversed the intestine was measured.

RESULTS. The accompanying table shows the results obtained. It clearly may be seen that ergotamine, prostigmine, atropine and ephedrine all have a pronounced effect on the motility of the small intestine. Cocaine had no inhibitory effect on intestinal motility.

Due to variations in the total length of the gut, even though matched pairs of dogs were used, it was necessary to use percentage figures, which have an elliptical distribution. This type of data does not lend itself accurately to statistical treatment unless all the values fall above 10 and below 90 per cent. The data which we used fell within these limits.

TABLE 1

Effect of autonomic agents on motility of small intestine in dogs

AGENT AND DOSAGE	TIME AFTER INTUBATION	EXPERIMENTAL			CONTROL			DIFFERENCE IN LENGTH TRAVERSED	FISHER'S "p"
		No. of animals	Av. length of gut	Length of gut traversed	No. of animals	Av. length of gut	Length of gut traversed		
	min.		*cm.*	%		*cm.*	%		
Ergotamine, 0.1 mgm./kgm.	15	12	205	74	12	224	52	+22	0.017
Progstigmine, 0.5 mgm./kgm.	15	7	294	81	7	281	43	+38	0.021
Atropine 1 mgm./kgm.	30	10	280	29	10	261	65	−36	<0.001
Ephredrine, 25 mgm./kgm.	30	9	269	40	9	219	75	−35	<0.001
Cocaine, 10 mgm./kgm.	30	7	237	63	5	211	70	− 7	>0.20

DISCUSSION. The results clearly show that the motility of the small intestine is appreciably affected by certain agents which influence the autonomic nervous system.

Ergotamine caused an increase in intestinal motility presumably by paralyzing the sympathetic nerves, which carry predominantly inhibitory fibers. Prostigmine, which also caused an increase in intestinal motility, inhibits the action of choline esterase and so promotes the accumulation of acetyl choline in the tissues.

Atropine and ephedrine both caused a decrease in intestinal peristalsis. The former agent acts by paralyzing the parasympathetic nerves which carry the visceromotor fibers, while ephedrine exerts its action by stimulating the receptor mechanism upon which ephedrine normally acts.

It is known clinically that following the administration of a spinal anesthetic agent, the small intestine is often more contracted than when inhalation anes-

thetics are given. The explanation is that the spinal anesthetic agent blocks the splanchnic nerves, so that the action of the parasympathetic nerves is left unopposed. In the laboratory, too, it may be demonstrated that if the splanchnic nerves are destroyed, the small intestine shows more vigorous peristalsis.

Cocaine had no appreciable effect on the motility of the small intestine. This agent acts by sensitizing the sympathetic nerve endings. It is noteworthy that cocaine showed no inhibitory effect on intestinal peristalsis, because it suggests that the conditions of the experiment produced no undue sympathetic stimulation.

It is of interest that ergotamine caused an increase of intestinal motility of 27 per cent and prostigmine 38 per cent, while on the other hand, atropine caused a decrease of 36 per cent and ephedrine 35 per cent. From these data the interpretation might be made that the inhibitory and motor nerves are rather evenly balanced, as far as their effect on the motility of the small intestine is concerned. It is recognized, however, that certain objections can be raised against the use of drugs in studying physiologic phenomena and it is questionable whether such an interpretation is justifiable. In studying the action of these various agents quantitatively on the motility of the small intestine the matter of dosage is, of course, an important item. The doses used, in our opinion, produced equivalent effects in either direction.

Since the agents which we used are known to affect the motility of the stomach as well as the small intestine, the question might be raised why the charcoal mixture was not placed directly into the duodenum. The material was put into the stomach because it was our desire to disturb the animal as little as possible. As previously mentioned the fact that cocaine produced no inhibitory effect on intestinal motility indicated that the experimental procedures did not excite the animals.

Three minutes after the charcoal mixture had been placed into the stomach the autonomic agent was administered; it took, of course, a little time for it to act; some of the charcoal mixture, therefore, had an opportunity to pass into the duodenum before the drug produced any appreciable effect on the stomach. It was the rate of progress of this portion of the mixture, which had entered the intestine, that interested us. We feel that we were not measuring the transport of the material through the stomach, but rather through the small intestine.

It has been shown in our laboratory that hunger contractions (2) and digestive peristalsis (3) are decreased by effective degrees of anoxia. It, too, has been shown that gastric emptying is delayed by anoxia (4). One logical explanation for these findings is that anoxic anoxia produces a stimulation of the sympathetic nerves.

It would be expected that the motility of the small intestine, which is known to have a similar innervation as that of the stomach, would likewise be affected by anoxic anoxia. In the dog it was observed that even a severe degree of anoxia (barometric pressure of 205.8 mm. Hg) produced no change in the motility of the small intestine. It will be seen in the accompanying table, however, that ephedrine produced a statistically significant decrease in motility. In view of

this latter finding it is difficult to understand why anoxic anoxia did not likewise decrease motility.

At the present time we can offer no explanation for this phenomenon. It might indicate that the stomach of the dog is more sensitive to anoxia than the small intestine, or on the other hand the inhibitory mechanism of the stomach may be more sensitive to anoxia and the excitatory mechanism of the intestine may be more sensitive to anoxia. Ephedrine, however, is known to have a marked inhibitory effect on the stomach (5) as well as the intestine.

SUMMARY

Matched pairs of unanesthetized dogs were given a powdered charcoal mixture by stomach tube. One animal served as a control and the other was given an agent which affected the autonomic nervous system. The following preparations were studied: ergotamine, prostigmine, atropine, ephedrine and cocaine. Appropriate doses of these drugs were administered intramuscularly and at the end of a specified time, the animal was sacrificed and the distance the charcoal mixture had traversed the small intestine was measured.

Ergotamine increased the motility of the small intestine 27 per cent and prostigmine 38 per cent; atropine decreased the motility 36 per cent and ephedrine 35 per cent. These figures were all statistically significant. Cocaine had no appreciable effect on the motility of the small intestine.

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THE EFFECT OF ADRENAL MEDULLECTOMY ON THE HEREDITARY DIABETES OF A STRAIN OF RATS[1]

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Received for publication March 10, 1944

Previous studies (1, 2) have shown that there exists in the Yale strain of rats an hereditary (3) type of "diabetes", manifested by a low tolerance to orally or intraperitoneally administered glucose. The etiology of this condition is not known, although some dysfunction of the anterior pituitary has been suggested as a causative factor (4). Experiments in this laboratory (5) have demonstrated that there is an impairment in the mechanism for either the depositing or the retaining of glycogen in the liver and tissues of the rats of the diabetic strain. Such a condition may conceivably result either from a failure of glycogenesis or from an increase in glycogenolysis. As is well-known, glycogenolysis is increased by epinephrine immediately after its injection. For this reason, together with the fact that rats of the Yale strain are hypersensitive to epinephrine (4), it appeared possible that an increased secretion of this substance might be involved in producing the low tissue glycogen values and the prolonged hyperglycemia observed in the glucose-injected diabetic animals (5). Possible support for such a view is the fact that the adrenal glands of male Yale rats are significantly heavier than those of male Wistar rats (6).

The present investigation was designed to determine the effect of demedullation of the adrenal glands on the glucose tolerance of diabetic Yale rats.

EXPERIMENTAL. Adult rats of the Connecticut Agricultural Experiment Station (Yale) strain weighing 250 to 400 grams were used. They were fed the stock colony ration (5). After a 16- to 18-hour fast, the animals were injected intraperitoneally with 350 mgm. anhydrous glucose (Pfanstiehl) per 100 grams body weight, using an 8.75 per cent solution measured from a graduated pipette. Blood sugar determinations were made by the Hagedorn and Jensen (7) method on blood obtained from a tail vein at regular intervals (see table 1) during a 5-hour period of observation. Glucose tolerance tests were carried out by this procedure on Wistar rats, on intact diabetic Yale rats, and on diabetic rats from which the medullas of the adrenal glands had been removed by the technique of Evans (8). The tolerance to glucose was determined in the latter group 18 to 24 days after demedullation, a time interval which should have permitted a regeneration of any traumatized cortical tissue (8). The tolerance to glucose was again determined in 12 of these animals 10 to 12 weeks later. The response to injected glucose was also determined in 6 of the demedullated rats following the administration of epinephrine which was injected subcutaneously in a dose

[1]Aided by a grant from the Graduate Council, Wayne University. A preliminary report was made before the American Society of Biological Chemists in Boston, April, 1942.

of 0.01 mgm. per 100 grams body weight immediately after the glucose was given and again 2½ hours later.

RESULTS. As is evident from the data given in table 1, the Wistar strain of rats showed a normal tolerance to the injected glucose, whereas the intact diabetic Yale rats exhibited the low tolerance previously demonstrated (1, 2). The demedullated diabetic rats, on the other hand, showed normal tolerance curves. This was found to be true in each of the 20 animals studied. The shift to a normal tolerance to injected glucose was particularly striking in 7 of the demedullated rats which, prior to medullectomy, had shown extremely high blood sugar values after glucose administration, the values exceeding 300 mgm. per cent 5 hours after the injection. The results, not included in the table, obtained in the tolerance tests made on the demedullated rats 10 to 12 weeks after operation were similar to those just described in all but 3 of the 12 animals. The blood sugar values on these 3 rats were slightly above normal at the end of

TABLE 1

*Blood sugar values of control rats and of demedullated rats following the administration of glucose**

GROUP	NO. OF RATS	BLOOD SUGAR† (MGM. PER CENT)				
		Initial	½ hr.	1 hr.	3 hr.	5 hr.
Intact Wistar rats	12	82 (78–92)	238 (228–256)	192 (177–237)	141 (117–163)	115 (85–134)
Intact Yale rats	25	98 (74–143)	240 (162–385)	212 (197–385)	193 (133–385)	219 (158–385)
Demedullated Yale rats	20	92 (81–105)	199 (145–247)	175 (130–235)	113 (56–145)	113 (52–145)
Demed. Yale + epinephrine	6	89 (83–96)	241 (231–274)	307 (250–385)	316 (160–385)	293 (215–385)

* 350 mgm. anhydrous glucose per 100 grams body weight, given intraperitoneally.

† The blood sugar values given are group averages with minimum and maximum individual values indicated in parentheses.

the 5-hour period, but still were not as high as had been found before medullectomy was performed.

The blood sugar levels of the demedullated rats given epinephrine after the glucose injection were extremely high, exceeding even those of the intact diabetic animals.

DISCUSSION. It would not be difficult to conclude from the foregoing data that a hyperactivity of the adrenal medulla may play a rôle in the diabetes of the Yale strain of rats. Certainly the removal of the medulla was followed by a normal tolerance to injected glucose and the administration of epinephrine reestablished a diabetic type of response. Also a hypersecretion of epinephrine might well produce the low liver and tissue glycogen values, the prolonged hyperglycemia, and perhaps other abnormalities seen in the Yale strain of rats (5). This explanation, likewise, would not be at variance with the observation (1, 5) that a low tolerance to glucose is not consistently found in all of the diabetic rats,

but that frequent alternations between normal and diabetic types of glucose tolerance curves occur in some animals. The foregoing observations may also be interpreted as indicating that there is not a serious impairment of the more fundamental mechanisms for regulating carbohydrate metabolism, as for example in the amount of insulin secreted. However, such a simple explanation may be unwarranted in view of the manifold factors affecting the metabolism of glucose in the animal organism. As is well-known, an adrenal cortical hormone exerts a profound effect on carbohydrate metabolism and it is possible that traumatization of cortical tissue may have affected the observed response, even though a presumably sufficient period for the regeneration of this tissue was allowed. This question merits further investigation.

SUMMARY

The removal of the adrenal medullas from rats showing a hereditary "diabetes" results in a normal tolerance to injected glucose.

The administration of epinephrine with glucose produces a diabetic type of tolerance curve in the demedullated animals.

These results indicate that there may be a relationship between the adrenal medulla and the diabetic tendency observed in the Yale strain of rats.

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THE EXCRETION OF UREA BY NORMAL SUBJECTS UNDER BASAL CONDITIONS

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Received for publication March 10, 1944

In two papers published in 1934 (1, 2) Pucher and his fellow-workers discussed the variation of various blood and urine constituents under basal conditions. Upon two of the subjects studied at that time data were available for a statistical study of urea and water excretion. The present paper contains a report of such a study.

The two subjects were young male adults, identified respectively as "AB" and "CD" in a previous publication (3). "AB" was 35 years old, 161 cm. tall, and weighed 51–55 kilos. His body surface, as calculated by the formula of DuBois (4) was 1.54 sq. m. and his basal metabolic rate, as an average of 260 determinations, was 32.86 ± 0.05 calories per m^2 per hour. "CD" was 28 years old, 170 cm. tall, and weighed 57–63 kilos. His body surface was 1.68 sq. m. and his basal metabolic rate as an average of 246 determinations was 33.65 ± 0.05 calories per m^2 per hour. Both men were in good health at the time when the studies were made and have continued to be so until the present time. Both were thoroughly trained in metabolism technique before the study was begun. There is every reason to believe that there was no cause for urinary retention by either subject.

The strict basal conditions under which these experiments were carried out have been described previously. The number of tests performed upon "AB" and "CD" were 40 and 43 respectively, or approximately one per week throughout the year from February 1926 to February 1927. The length of the different test periods varied somewhat; as shown in table 1 the average was somewhat over 2 hours. The blood for analysis was collected from an arm vein without stasis at the end of the metabolic period. This was done to avoid interference with the basal metabolism determination which formed a part of each study (3). The bladder was emptied voluntarily as completely as possible just before and at the end of the approximately 2-hour period. Because each subject was studied under strictly controlled basal conditions, and had not eaten or drunk for the preceding 12 to 14 hours, it does not seem probable that the concentration of urea in the blood could have varied significantly during the period of the collection of the urine samples.

The usual statistical constants derived from the different findings upon the two subjects are given in table 1. These include the concentration of urea in the blood and urine, the rate of urea excretion, and the volume of urine formed per minute. Various ratios between the blood and urine urea are also given. These are: concentration of urea in urine/concentration in blood; rate of excretion

of urea/blood urea concentration ("maximum clearance") (5); the ratio $\sqrt{\text{urine volume}}$ X concentration of urea in urine/blood urea ("Standard Clearance") (5) and, for subject "AB" a "combined clearance" in which the standard clearance was calculated for periods when the urine volume was under 2 cc. per minute and the maximum clearance for periods with a greater urine volume, and the 40 values so obtained combined (6).

It is evident from the table that the only conspicuous difference between these subjects was that "AB" excreted a volume of urine more than 2.5 times as great

TABLE 1

Statistical constants

SUBJECT	NUMBER	DETERMINED	RANGE	MEDIAN	MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION
							per cent
A. B.	40	Duration (min.)	90 -186	136 ±3	137 ±2	18 ±1	14 ±1
		Blood urea concentration (mgm./100 cc.)	8.7 - 14.3	11.6 ±1.6	11.8 ±1.3	1.40 ±0.11	11.9 ±0.9
		Urine volume (cc./min.)	0.89- 3.07	1.84 ±0.25	1.86 ±0.20	0.493 ±0.037	26.3 ±2.0
		Urine urea excretion (mgm./min.)	3.96- 7.43	5.75 ±0.75	5.58 ±0.60	0.814 ±0.061	14.5 ±1.2
		Urine urea concentration (mgm./cc.)	1.9 - 6.3	2.8 ±0.4	3.24 ±0.35	1.09 ±0.08	34.3 ±2.6
		Maximum clearance (cc./min.)	36 - 56	48 ±6	47.4 ±5.1	4.60 ±0.35	9.8 ±0.8
		Standard clearance (cc./min.)	25 - 53	34 ±5	35.7 ±3.8	5.44 ±0.41	15.0 ±1.2
		Combined clearance (cc./min.)	30 - 56	40 ±5	42.0 ±4.5	7.36 ±0.56	17.6 ±1.4
		Ratio—urine urea:blood urea	17 - 56	26 ±4	27.1 ±2.9	8.19 ±0.62	30.4 ±2.3
C. D.	43	Duration (min.)	91 -238	164 ±3	167 ±2	22 ±2	13 ±1
		Blood urea concentration (mgm./100 cc.)	7.8 - 13.1	10.6 ±1.4	10.6 ±1.1	1.21 ±0.09	11.3 ±0.8
		Urine volume (cc./min.)	0.32- 1.22	0.67 ±0.12	0.72 ±0.08	0.233 ±0.017	32.0 ±2.3
		Urine urea excretion (mgm./min.)	3.91- 8.57	5.46 ±0.70	5.45 ±0.56	0.908 ±0.066	18.4 ±1.3
		Urine urea concentration (mgm./cc.)	4.1 - 15.0	8.3 ±1.9	8.20 ±1.53	2.40 ±0.18	29.3 ±2.1
		Maximum clearance (cc./min.)	35 - 69	52 ±6	51.6 ±5.3	8.77 ±0.64	16.9 ±1.2
		Standard clearance (cc./min.)	45 - 81	60 ±8	62.0 ±5.8	9.73 ±0.71	15.6 ±1.2
		Ratio—urine urea:blood urea	48 -142	73 ±9	76.3 ±6.8	21.1 ±0.9	27.6 ±2.0

as did "CD." The rates of excretion of urea were almost identical, and the average blood urea concentrations differed from each other by only 11 per cent. It is evident also that the degree of constancy of the blood urea and of the excretion of urea in the urine was very high in each instance, but that the rate of excretion of fluid, and consequently of the concentration of urea in the urine showed a much greater degree of variability. A further discussion of the figures will be given later.

In table 2 the correlation coefficients between the different factors are given.

These coefficients were qualitatively alike (possessed the same sign) for both subjects in all instances, but in several of the comparisons where the correlation found upon one subject was rather small, but statistically significant, the significance of that upon the other man was questionable.

Some of the relationships shown in the table seem to the authors to deserve comment. The blood concentration showed significant positive correlations with both the excretion rate and the concentration of urea in the urine. In both subjects the latter correlation was the closer one, but in neither was the difference between the coefficients significant.

In table 3 there is given further data bearing upon the relationship between blood and urine urea. In this table partial correlation coefficients of the first

TABLE 2

Correlation coefficients

SUBJECT	SECONDARY VARIABLES	PRIMARY VARIABLES			
		Urine volume	Rate of urea excretion	Blood urea concentration	Urine urea concentration
A. B. 40 tests	Urine volume		+0.103 ±0.106	−0.161 ±0.103	−0.823 ±0.034
	Rate of urea excretion	+0.103 ±0.106		+0.370 ±0.092	+0.393 ±0.090
	Blood urea concentration	−0.161 ±0.103	+0.370 ±0.092		+0.473 ±0.083
	Urine urea concentration	−0.823 ±0.034	+0.393 ±0.090	+0.473 ±0.083	
	Maximum clearance	+0.424 ±0.088	+0.490 ±0.081	−0.202 ±0.102	−0.089 ±0.106
	Standard clearance	−0.686 ±0.057	+0.356 ±0.093	+0.090 ±0.106	+0.918 ±0.017
	Combined clearance	+0.438 ±0.086	+0.388 ±0.090	−0.070 ±0.106	−0.029 ±0.107
	Ratio—urine urea concentration:blood urea concentration	−0.852 ±0.028	+0.142 ±0.103	+0.144 ±0.103	+0.918 ±0.017
C. D. 43 tests	Urine volume		+0.322 ±0.092	−0.394 ±0.087	−0.852 ±0.029
	Rate of urea excretion	+0.322 ±0.092		+0.319 ±0.092	+0.094 ±0.102
	Blood urea concentration	−0.394 ±0.087	+0.319 ±0.092		+0.417 ±0.085
	Urine urea concentration	−0.852 ±0.029	+0.094 ±0.102	+0.417 ±0.085	
	Maximum clearance	+0.615 ±0.064	+0.705 ±0.053	−0.425 ±0.084	−0.243 ±0.097
	Standard clearance	−0.449 ±0.082	+0.324 ±0.092	−0.105 ±0.102	+0.689 ±0.054
	Ratio—urine urea concentration:blood urea concentration	−0.772 ±0.042	+0.011 ±0.103	+0.060 ±0.103	+0.917 ±0.016

order are given. These were calculated by the method described by Pearl (7). They include certain relationships when the blood urea and urine volume were mathematically rendered constant. The table appears to show that under the basal conditions maintained during these experiments there was somewhat closer agreement between the concentration of urea in the blood and in the urine than between the blood urea and the rate of urea excretion, but the difference is not a very marked one.

As a result of the relationship between the blood urea concentration on the one hand and the urinary excretion and concentration of that compound on the other, it is evident that ratios between the blood urea and these two other factors should show less variation than do the urine measurements themselves. Reference to table 1 shows that this was the case. The ratio urine urea concen-

tration/blood urea and the maximum clearance both show lower coefficients of variation than do the urea concentration and the urea excretion.

Table 2 shows that in both subjects there was a slight negative correlation between the urine volume and the concentration of urea in the blood. However, the relationship found in one of the two instances (subject "AB") was not significant. These results seem to show that variations in the concentration of urea entering the glomerular filtrate did not cause significant variations in the amount of fluid retained in the tubules in these experiments. It seems most probable to the authors that this negative correlation is found because the concentration of urea in the blood stream and the volume of urine excreted both vary with the amount of water in the blood stream, but the relationship is so slight than no emphasis can properly be placed upon any explanation of it.

TABLE 3

Partial correlation of coefficients of the first order

SECONDARY VARIABLE	PRIMARY VARIABLE					
	Subject "A. B."			Subject "C. D."		
	Urine volume held constant					
	Blood urea concentration	Urea excretion	Urine urea concentration	Blood urea concentration	Urea excretion	Urine urea concentration
Blood urea concentration		+0.513 ±0.079	+0.744 ±0.048		+0.394 ±0.087	−0.859 ±0.027
Maximum clearance	−0.252 ±0.100	+0.679 ±0.058		−0.150 ±0.101	+0.346 ±0.091	
Ratio—urine urea concentration: blood urea concentration	−0.418 ±0.088		+0.778 ±0.042	+0.014 ±0.103		+0.728 ±0.049
	Blood urea concentration held constant					
Urine volume		+0.514 ±0.079	−0.842 ±0.031		+0.178 ±0.100	−0.859 ±0.027

Both subjects showed positive correlations between the urine volume and the rate at which urea was excreted in the urine, but neither coefficient was large, and that found upon the subject who excreted the largest volume of fluid per minute ("AB") did not possess statistical significance. In marked contrast with these figures were the high negative correlations found between the urine volume and the concentration of urea in the urine. To a very large extent the excretion of urea and fluid appear to have been independent of one another in these experiments, although this independence was evidently not complete. Reference to table 3 in which coefficients of the first order are given shows that when the blood urea was mathematically held constant the relationship between the urine volume and the excretion and concentration of urea were essentially the same as those shown by the coefficients of the zero order just discussed.

It seems to the authors that the relationships of the different factors to the

values of the ratios based upon them is of considerable interest. In general the values of a ratio will show a positive correlation with the value of the numerator and a negative correlation with the value of the denominator. If the ratio is a perfect one these correlations will be equal in magnitude. The relationships between the ratios and their components shown in table 2 are markedly irregular. There were marked positive correlations with the numerators—the excretion and concentration of urea—but the negative correlations with the denominators—the blood urea concentration—were only slight when they were present, and in some instances these correlations were positive. This, it seems to the authors, means that there were variations in the excretion of urea which were not related to the measured variations in the concentration of urea in the blood. In table 3 are given the same relationships when the urine volume was held constant. These results imply the existence of a high degree of independence of urea excretion with both the urine volume and the blood urea concentration, for a significant positive correlation between these fractions and their numerators exists, while only one of the four ratios shows a significant negative correlation with the blood urea, and the mathematical value of this negative ratio is significantly lower than is the positive correlation with the numerator. These figures show that under these basal conditions the excretion of urea showed variations which were to a considerable degree independent of both the blood urea and the urine volume. It seems most probable that these variations were due to variations in the rate of reabsorption from the tubules.

The ratios included in this discussion have been proposed as aids in evaluating the function of the kidneys under varying conditions. It seems worthwhile to discuss them briefly in reference to the two subjects studied.

Table 1 shows that a comparison based upon the values of the ratio urine urea concentration/blood urea concentration would have been very unsatisfactory. Even under basal conditions this ratio showed a very great degree of variability in each subject, and marked differences between the apparent kidney efficiency of two normal men, for subject "CD" would apparently have a kidney function over twice as great as that of subject "AB." A comparison based upon the values of the maximum clearance—the ratio: rate of urine excretion/blood urea concentration—would obviously be much more satisfactory. As already pointed out, the value of this ratio upon each subject showed a very high degree of constancy. Only the coefficients of variation of the blood urea concentrations were as small as were those of this ratio. Furthermore the apparent kidney efficiency of the two subjects, if based upon the values of these ratios, would be almost identical, as would be expected. If a correction of the two figures based upon the body surface of the subjects were introduced into the calculations, as recommended by McIntosh, Möller and Van Slyke (8) the agreement between the results would be even closer than is that shown, for the subject with the higher clearance also possessed the larger body surface. Both values were somewhat lower than are the values usually accepted as normal.

Möller, McIntosh and Van Slyke have (5) recommended the use of the standard clearance—values of the ratio $\sqrt{\text{urine volume}} \times$ concentration of urea in

urine/concentration of urea in blood—as a basis for comparing results upon subjects who excrete less than 2 cc. of urine per minute. All of the specimens obtained from subject "CD" and more than half (25 out of 40) of those of subject "AB" fell within this range. Upon subject "CD" the value of the standard clearance appears to have served as a better measurement of kidney function than did that of the maximum clearance. The variability of the former clearance was slightly smaller than that of the latter one, although the difference was not significant. The actual value of the clearance was much greater, and approached more nearly to the values which have been considered normal for such studies. It is worth noting that in the studies of this subject there was a significant positive correlation between the urine volume and the rate of excretion of urea. Upon subject "AB" it is evident that the standard clearance was a much less satisfactory measure of kidney function. The values of the ratio showed a high degree of variability, and the mean value was even lower than was that of the maximum clearance. Because some of the samples voided by this man were above and others lower than 2 cc. per minute a "combined clearance" was calculated for this subject. This has been defined already. This clearance did not apparently afford as satisfactory a measure of the kidney function of this patient as did the maximum clearance. The mean value was approximately the same as that of the maximum, and the variability even greater than that of the standard clearance. It should be noted that this subject did not show a significant correlation between the urine volume and the rate of urea excretion, and so it is impossible to determine the augmentation limit (9, 5) in this instance. It seems quite certain that it was not 2 cc. per minute, and it is upon this figure that the calculation of what has been called here the "combined clearance" is based.

SUMMARY

The excretion of urea by two normal male subjects under basal conditions has been discussed. The concentration of urea in the blood and the rate of urea excretion were quite constant, and nearly the same in both of these subjects, but the water excretion and the concentration of urea in the urine fluctuated markedly in both of them. There was a positive correlation of the blood urea concentration with both the urea excretion rate and the concentration of urea in the urine; this relationship was somewhat closer with the concentration than the excretion, but the difference was not marked. There was a slight negative correlation between the blood urea concentration and the urine volume. While there was a slight positive correlation between the urine volume and the rate of urea excretion, the relative independence of urea and fluid excretion was much more marked. Evidence was found that under these basal conditions the excretion of urea to some extent varied independently of variations in blood urea and urine volume. In these two cases the ratio: rate of urea excretion/concentration of urea in the blood appeared to be a somewhat better basis for estimating kidney function than did other ratios studied. It appeared probable that this was due to the lack of correlation between urea excretion and urine volume shown by one of the subjects.

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THE EFFECT OF FASTING ON THE BLOOD SUGAR CURVE OF THE EVISCERATED RAT[1, 2]

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Received for publication March 13, 1944

Roberts et al. (1944) have recently found that the non-nephrectomized, eviscerated rat, previously force-fed a diet rich in fat, is able to maintain a higher concentration of blood sugar and to survive longer than similar animals maintained on equicaloric amounts of a high carbohydrate diet.

The energy-producing substances available during early fasting are chiefly fat and some protein (Kingdom et al., 1942). The present investigation was undertaken to determine if fasting would affect the eviscerated rat in a manner comparable to the feeding of fat. As will be noted below, differences as well as similarities exist in the two instances. The mechanisms involved in each case, therefore, are not necessarily the same.

METHODS AND MATERIALS. Young male rats, obtained from Sprague-Dawley, Inc., and weighing 60 to 70 grams, were prepared for eventual evisceration by complete ligation of the vena cava above the renal veins. They were allowed to grow to adulthood on a diet of Purina "Whelping Fox Chow" fed libitum. When the animals had attained a weight of about 300 grams, they were separated into three groups having similar weight ranges. One group was fasted approximately four days, another two days, and the third group was allowed access to food until operation.

Complete abdominal evisceration, with or without simultaneous nephrectomy, was performed in a manner described previously (Reinecke, 1943). Blood samples were obtained from the clipped end of the tail immediately preceding and following the operation and at frequent intervals thereafter. In the case of the non-nephrectomized eviscerated rats a sample was taken from the right ventricle at death; heart samples have previously been shown to be comparable in blood sugar content to simultaneous tail samples at this time (Roberts et al., 1944). The amount of blood drawn in most cases was 0.05 ml. Duplicate determinations of blood sugar were made on tungstic acid filtrates by a microferricyanide method (Reinecke, 1942).

All animals were given a subcutaneous injection of 2 cc. of physiological saline per 100 grams of body weight immediately before they were anesthetized preparatory to operation. Those non-nephrectomized animals that were still alive nine hours after ligation of the coeliac and mesenteric arteries were further injected

[1] Aided by grants from the committee on Scientific Research of the American Medical Association and from the John and Mary R. Markle Foundation.

[2] The authors wish to express their appreciation for the encouragement and advice given by Dr. L. T. Samuels.

subcutaneously with 2 cc. of physiological saline per 100 grams eviscerated body weight.

The time required for performing the operation was considerably longer in the case of the nephrectomized animals, because a cannula was placed in the external jugular vein incidental to other studies carried out on these animals subsequent to the time indicated by the last plotted points on their respective curves. Cannulation was carried out prior to the evisceration procedure.

RESULTS. *Effect of fasting on the non-nephrectomized, eviscerated rat.* Inspection of the blood sugar curves of the non-nephrectomized animals (fig. 1) reveals a clear difference between fed and fasted animals. There is, however, no definite difference between the animals fasted two and four days.

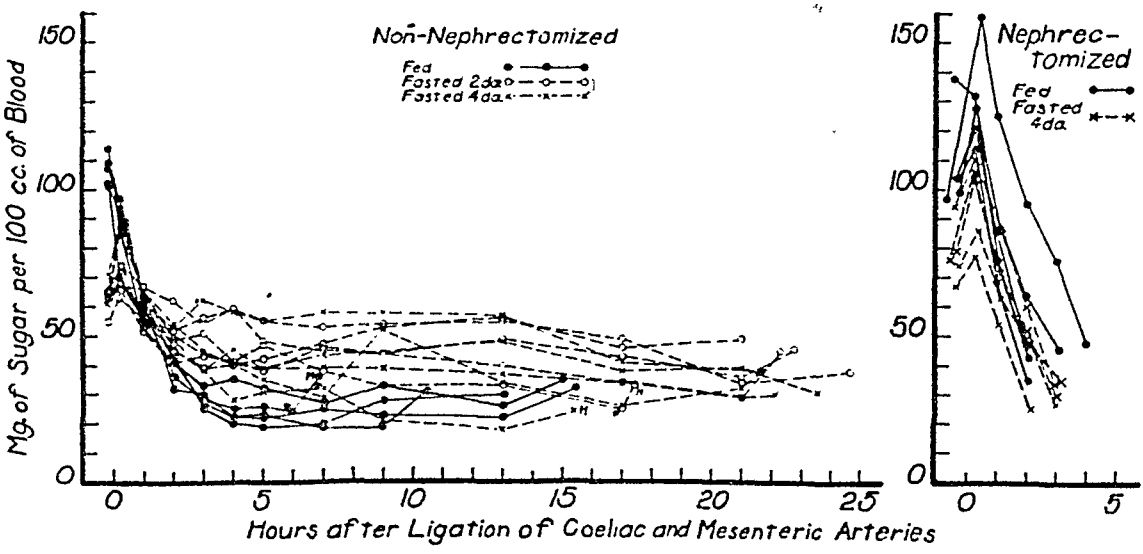

Fig. 1. The effect of fasting on the blood sugar curve of the eviscerated rat. A letter *H* near the final plotted point on a curve indicates that the corresponding animal suffered a significant hemorrhage. An asterisk alongside the same curve shows when the hemorrhage was first noticed.

It is of particular interest to consider the section of the curves between one hour and three hours after ligation of the arteries. This period demonstrates a difference between the fed and fasted groups which appears to be absent in animals with kidneys removed in addition to abdominal evisceration. At one hour the ranges of blood sugar values of fed and fasted animals are similar; at three hours, however, the blood sugar values of the fasted animals are definitely higher than those of the fed animals.

The remainder of the blood sugar curves (subsequent to 3 hrs.) indicates that the animals which had been fasted were able to stabilize their blood sugar at higher levels than those which had been fed. Only one of the animals that had been fasted exhibited blood sugar concentrations within the low range of the fed rats. This animals suffered a significant loss of blood which in itself is sufficient to cause the development of hypoglycemia (Reinecke, 1943).

The length of survival after ligation of the coeliac and mesenteric arteries was

much less for the fed animals than for those that had been allowed to fast. Excluding the animals that had suffered significant hemorrhage, those that had been fed survived only $10\frac{1}{2}$, 13, 15 and $15\frac{1}{2}$ hours, as compared to 21, $22\frac{1}{4}$, $22\frac{1}{4}$ and $24\frac{1}{4}$ hours for those that had fasted two days, and $21\frac{1}{2}$, $21\frac{3}{4}$, 22 and $23\frac{1}{2}$ hours for those that had fasted four days. Death in convulsions occurred in all the fed animals, but in none of the fasted rats.

Effect of fasting on the nephrectomized, eviscerated rat. The most salient feature of the curves of the nephrectomized animals (fig. 1) is that all segments subsequent to the points plotted at the end of the operative period tend to have the same slope irrespective of whether the animals had been fed or fasted.

It may also be noted that there was a more marked tendency for the blood sugar concentration to increase during the operative period in this study than in the experiments on the non-nephrectomized rats. It may be that this was merely a result of the longer period of ether anesthesia incidental to cannulation of the external jugular vein before exclusion of the liver from the circulation and evisceration.

Discussion. The blood sugar method used in this experiment measures some non-fermentable reducing substances as sugar (Reinecke, 1943). This complication must be borne in mind as a limitation to the interpretation of the data. In the case of animals previously maintained on a high fat or high carbohydrate diet it was found that the non-fermentable reducing substances increased with time after evisceration, but did not differ significantly between the groups on the different diets (Roberts et al., 1944).

The similarity of the blood sugar curves of fed and fasted nephrectomized, eviscerated rats stands in sharp contrast to the difference found in a comparable portion of the curves of the corresponding group of animals not subjected to nephrectomy. This may mean that the kidney is involved in the readjustment of metabolism that is induced by fasting.

The results obtained on the non-nephrectomized, eviscerated rats support the findings of Drury (1935) and of Bergman and Drury (1937). These workers found that it was necessary to inject glucose less rapidly to maintain the initial blood sugar level of the fasted non-nephrectomized, eviscerated rabbit than similarly prepared animals that had been fed prior to evisceration.

The lack of a clear-cut difference between the two and four day fasted animals in the present study suggests that the metabolic readjustments involved in these experiments are completed by two days of fasting. The fact that the differences between the group that had been fed and those that had fasted persisted throughout the experimental period, on the other hand, may imply that at least several hours are required for these readjustments. On the other hand, Drury (1935) has observed a decreased utilization of glucose in the fed, non-nephrectomized, eviscerated rabbit after about 24 hours of survival which might possibly be due to such a readjustment occurring within the experimental period.

The finding that fasting as well as the proportion of fat to carbohydrate in the diet markedly affects the behavior of the non-nephrectomized, eviscerated rat is a warning that the state of nutrition must be carefully considered in studies on this preparation.

SUMMARY

Fasting has been found to have a marked effect on the blood sugar concentration and the length of survival of the non-nephrectomized, eviscerated rat. Blood sugar was maintained at a significantly higher level and survival time was twice as long in rats fasted two or four days as compared to rats fed to time of operation. Nephrectomy appeared to eliminate some of these differences.

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THE EFFECT OF INTRAHEPATIC PRESSURE ON BILE RESORPTION DURING OBSTRUCTIVE JAUNDICE

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Received for publication March 20, 1944

Vascular changes in the liver produced by surgical procedures or by the injection of drugs have been studied from the standpoint of bile secretion (1), portal venous pressure (2) and liver volume (3). These investigations have resulted in advances of our knowledge of the intrahepatic circulation, the hepatic portal anastomosis and the relationship of the hepatic nerves to the liver capillaries.

In this laboratory, the absorption of bile during obstructive jaundice was studied under conditions in which the blood supply of the liver was little disturbed. It was concluded that both the blood and the lymph participated in the absorption of bile and that the quantity of resorbed bilirubin depended on intrabiliary pressure (4). In another series of experiments it was shown that the main absorptive function could be taken over by the blood when the lymphatic pathway was obstructed (5). The present experiments were designed to study the effect of vascular changes in the liver on bile resorption during obstructive jaundice. This method was not previously employed in the experimental approach to the jaundice problem as far as the authors could determine.

METHOD OF STUDY. The following procedures were performed on thirty-six dogs. Each animal was anesthetized by an intravenous injection of pentobarbital sodium, 50 mgm. per kilogram. Following this an endotracheal tube was passed and artificial respiration was administered by an insufflation machine. The thoracic lymph duct was isolated in the neck, severed from its venous termination and cannulized. In the abdomen, the cystic duct was ligated and the common bile duct was intubated with a glass cannula. The latter was connected by a short piece of rubber tubing attached to a small graduated flask or reservoir. The biliary ductal system was then filled with the animal's own bile obtained by aspiration from the gall bladder and diluted with two volumes of normal saline solution. Each experiment was conducted under a constant hydrostatic pressure. This was done by raising the bile containing reservoir to 300 mms. above the level of the porta hepatis. The meniscus of the bile solution in the reservoir was the fixed point from which any fluctuation in biliary pressure was measured.

In addition to the above mentioned procedures, vascular or neuro-vascular changes in the liver were produced by a variety of surgical methods. The animals in the entire series may be classified into four groups. Group I. Hepatic artery ligated. Group II. Hepatic artery denervated. Group III. a. Eck fistula. b. Eck fistula with hepatic artery ligated. c. Reversed Eck fistula. Group IV. Hepatic veins obstructed.

An initial preoperative sample and several postoperative samples of blood and lymph were taken at regular intervals for quantitative bilirubin determination by the van den Bergh method. Lymph was collected at regular periods corresponding in time with each blood sample. The total output of lymph was measured and noted. The intake of bile by the liver represented the total amount of bile solution added to the graduated reservoir during the experimental period. Bile solution was added only when the intrabiliary pressure fell below the standard experimental pressure.

RESULTS. *Group I.* The hepatic artery was ligated without injury to the periarterial plexus of the nerves. Ligation of this artery did not result in any visible change in the gross appearance of the liver. During the period of obstructive jaundice, the absorption of bilirubin into the blood was slow and

TABLE 1

The effect of ligation of the hepatic artery on the concentration of bilirubin in the blood and lymph during obstructive jaundice

Bilirubin (mgm. per cent)

EXPT.	SAMPLE	INITIAL	10 MIN.	20 MIN.	40 MIN.	60 MIN.	80 MIN.	100 MIN.	120 MIN.
1	Blood	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Lymph	0.00	0.00	0.00	0.09	0.16	0.39	0.51	0.89
2	Blood	0.00	0.04	0.08	0.13	0.16	0.19	0.23	0.46
	Lymph	0.00	0.06	0.10	0.19	0.25	0.38	0.42	0.57
3	Blood	0.00	0.00	0.13	0.18	0.22	0.24	0.22	0.36
	Lymph	0.00	0.00	0.20	0.53	0.82	0.98	1.00	1.16
4	Blood	0.00	0.00	0.00	0.12	0.26	0.42	0.48	0.53
	Lymph	0.00	0.10	0.15	0.28	0.46	0.63	0.92	1.06
5	Blood	0.00	0.00	0.00	0.09	0.10	0.18	0.34	0.58
	Lymph	0.00	0.05	0.12	0.22	0.26	0.30	0.48	0.72

quantitatively small. Bilirubin resorption in the lymph was more rapid and increased progressively in amount. The results obtained were similar to those obtained in a series of earlier experiments (4) in normal dogs in which there was no disturbance of the blood supply of the liver (see table 1).

Group II. Denervation of the liver was performed by the technique recommended by Lundberg (6). During the period of obstruction bilirubin appeared quickly both in the peripheral blood and lymph and a constant slow increase was noted. The values for bilirubin in the blood and lymph exceeded those of the previous experiments. The intake of bile averaged about 8.3 cc. Denervation of the liver did not appear to affect the flow of lymph or its output (see table 2).

Group IIIa. By means of the Eck fistula the portal blood stream was diverted from the liver into the general venous circulation. This procedure affected the process of absorption during obstructive jaundice. As a result bilirubin was

detected only in traces or in small quantities in the blood. However the resorption of bilirubin into the lymph was not depressed to the same extent. The intrabiliary pressure tended to fall below the standard level and bile solution was frequently added to the reservoir. The intake of bile (23 cc. av.) exceeded that

TABLE 2

Effect of denervation of hepatic artery on biliary resorption during obstructive jaundice

Bilirubin (mgm. per cent)

EXPT.	SAMPLE	INITIAL	10 MIN.	20 MIN.	40 MIN.	60 MIN.	80 MIN.	100 MIN.	120 MIN.
6	Blood	0.00	0.25	0.28	0.41	0.48	0.61	0.81	0.83
	Lymph	0.00	0.42	0.58	0.78	0.83	0.92	1.02	1.14
7	Blood	0.00	0.35	0.44	0.73	0.73	0.60	0.83	1.01
	Lymph	0.00	0.39	0.54	0.92	1.32	1.56	1.81	2.12
8	Blood	0.00	0.20	0.39	0.68	0.94	1.20	1.30	1.52
	Lymph	0.00	0.28	0.49	0.83	1.32	1.68	2.04	2.14
9	Blood	0.00	0.37	0.35	0.41	0.63	1.37	1.93	2.00
	Lymph	0.00	0.23	0.81	1.30	2.40	3.10	3.26	3.42
10	Blood	0.00	0.49	0.35	0.53	0.76	1.08	1.34	1.46
	Lymph	0.00	0.52	0.58	0.78	1.50	2.20	2.42	2.84

TABLE 3

The concentration of bilirubin (average values) in the blood and lymph in Eck fistula dogs during obstructive jaundice. Initial concentration of bilirubin in blood and lymph was 0.00

Bilirubin (mgm. per cent)

EXPT.	TYPE OF EXPERIMENT	SAMPLE	10 MIN.	20 MIN.	40 MIN.	60 MIN.	80 MIN.	100 MIN.	120 MIN.
11–20	Eck fist.	Blood	0.00	0.00	0.11	0.31	0.44	0.61	0.79
		Lymph	0.00	0.18	0.21	0.66	0.93	1.20	1.60
21–26	Eck fist. hep. a. lig.	Blood	0.00	0.00	0.06	0.12	0.12	0.26	0.32
		Lymph	0.05	0.05	0.18	0.48	0.52	0.73	0.91
27–31	Reversed Eck fist.	Blood	0.18	0.31	0.62	0.92	0.96	1.23	1.46
		Lymph	0.24	0.54	0.94	1.48	1.48	1.93	2.06

of the previous experiment. The rate of flow and the output of lymph (65 cc. av.) were also increased (see table 3).

Group IIIb. In this group of dogs, in addition to diversion of the portal blood stream into the inferior vena cava by means of the Eck fistula, the hepatic artery was ligated. This resulted in a diminution in the size of the liver and affected its absorptive mechanism during the period of obstructive jaundice. Bilirubin was not detected in either the blood or lymph in two experiments. In the other

three only small amounts of bile pigment were found in either the blood or lymph. In this group also there was a large intake of bile solution (28 cc. av.) and a marked output of lymph (53 cc. av.) (see table 3).

The same effects were obtained in experiments in which in association with evisceration of the gastro-intestinal tract the portal vein and hepatic artery were ligated. The degree of bilirubin resorption into the blood and lymph, the intake of bile and the output of lymph were similar to that described for group IIIb. However the extensive operative procedure employed in this experiment introduced the added non-physiologic factors of shock and hemorrhage.

Group IIIc. Reversal of the Eck fistula brought both portal and caval blood to the liver. The capacity for bile resorption was increased during this period of visceral engorgement and obstructive jaundice. Biliburin was noted quantitatively in the blood and lymph in all the experiments. In two cases bilirubin appeared in unusually high concentration in the lymph. The flow of lymph was rapid and profuse (82 cc. av.) but the total intake of bile averaged 18 cc. (see table 3).

TABLE 4

Effect of obstruction of the hepatic veins on the concentration of bilirubin in the blood and lymph during obstructive jaundice

Bilirubin (mgm. per cent)

EXPT.	SAMPLE	INITIAL	10 MIN.	20 MIN.	40 MIN.	60 MIN.	80 MIN.	100 MIN.	120 MIN.
32	Blood	0.00	0.31	0.72	0.83	0.92	1.30	1.86	2.46
	Lymph	0.00	0.56	0.88	1.04	1.23	1.52	2.14	3.26
33	Blood	0.00	0.12	0.18	0.30	0.86	1.14	1.36	1.80
	Lymph	0.00	0.16	0.52	0.71	1.06	1.45	1.83	2.06
34	Blood	0.00	0.00	0.11	0.26	0.53	0.79	0.92	1.20
	Lymph	0.00	0.08	0.20	0.38	0.88	1.00	1.28	1.56
35	Blood	0.00	0.10	0.30	0.46	0.62	0.94	1.24	1.50
	Lymph	0.00	0.24	0.46	0.74	1.00	1.24	1.38	1.76
36	Blood	0.00	0.18	0.26	0.50	0.74	1.06	1.20	1.46
	Lymph	0.00	0.20	0.55	0.68	0.88	1.24	1.63	2.00

Group IV. The hepatic veins were obstructed by constriction with a fine rubber tourniquet. In two experiments a cord ligature was used. Complete obstruction was not obtained but produced changes sufficiently significant to prove this method effective. In this group three out of eight dogs died before the completion of the experiment. In the successful experiments the liver was observed to assume a dark purplish color and appeared markedly engorged. After obstruction of the bile duct bilirubin appeared in significantly large quantities both in the blood and lymph. Little bile solution was needed for the

reservoir (4.2 cc. av.). The output of lymph was greater than in the previous group and averaged 97 cc. for the experimental period (see table 4).

DISCUSSION. The interpretation of the results obtained in these experiments may be based on the relationship of bile resorption and intrahepatic blood pressure to each other during the period of obstructive jaundice. Involved in the latter consideration are such factors as the minute volume flow of blood through the liver, filtration pressure, perilobular pressure, lobular pressure and bile capillary pressure. Fundamental observations on the relationship of intrahepatic pressure to bile secretion have already been made by Tanturi and Ivy (1).

In the present experiments it was found that bile resorption depended on intrahepatic blood pressure. The absorption of bile was markedly diminished when the portal vein was ligated and the main blood supply to the liver was interrupted. This involved a fall in the internal blood pressure of the liver as a result of which the blood capillaries and delicate sinusoids became compressed by the sustained (300 mm. H_2O) pressure from the intrabiliary radicles (bile capillaries). In turn the bile capillaries were more easily distensible and filled with bile from the reservoir. Experimental procedures in which both the hepatic artery and portal vein were ligated yielded similar results and emphasized further the importance of the intrahepatic pressure on the absorptive mechanism. However obstruction of the hepatic artery alone did not impair the resorptive capacity of the liver during jaundice since the internal visceral pressure was adequately maintained by the portal blood stream. The reversed condition of increased intrahepatic pressure obtained by obstruction of the hepatic veins resulted in an increased absorption of bilirubin during the period of jaundice. The latter may be accounted for on the basis of alteration of permeability of the hepatic cells probably caused by anoxia and resulted in a rapid diffusion of bilirubin into the blood and lymph. Denervation of the liver resulted in an increased secretion of bile in a system already obstructed at 300 mm. H_2O pressure. As previously reported, bilirubin resorption increased with a rise in intrabiliary pressure during the obstructive period (4).

An increased flow of lymph was observed in all cases in which the portal vein was ligated. This should be regarded as the usual concomitant of venous obstruction and was adequately described by Starling (6) many years ago. The increased flow of lymph occurred also after obstruction of the hepatic veins and could be accounted for on the same basis as the latter.

SUMMARY

1. Vascular and neurovascular changes in the liver modified the resorption of bile during obstructive jaundice.

2. A decrease in intrahepatic vascular pressure reduced the absorptive capacity of the liver in jaundice.

3. An increase in intrahepatic vascular pressure resulted in significant and rapid concentration of bilirubin in the blood and lymph.

4. The resorption of bile depended on intrahepatic vascular pressure as well as intrabiliary pressure.

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ACOUSTIC ALTERATIONS OF POST-CONTRACTION HYPERTONUS IN LIMB MUSCLES OF NORMAL MAN

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Received for publication March 11, 1944

The author has shown (1) that the temporary hypertonus of extensor or flexor muscles of the limbs, which develops following relaxation of voluntary isometric contraction of the muscles against resistance, serves as a basis for the study of tonic neck, labyrinthine, eye, nociceptive and other reflexes which can be shown to "catch on" and to affect, as in a decrebrate animal, the augmented stretch reflexes of normal human subjects.

The present study involves observations of the effects of acoustic stimuli upon these same hypertonic states of extensors and flexors of normal human limbs.

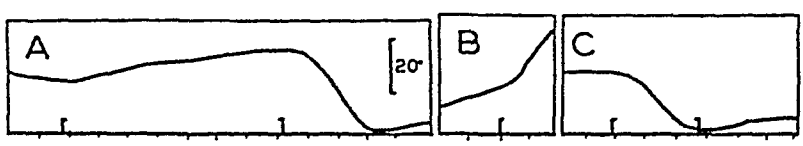

Fig. 1. Effects of 60 cycle tone, applied monaurally, upon extensor hypertonus of the arm. The upper record measures the angle of abduction of the arm, which increases with the tonus. Sound is signalled between brackets in the lower line on which time intervals of one second are indicated. A. A declining extensor tonus is redeveloped by sound in the contralateral ear. B. Development of tonus, immediately following cessation of voluntary contraction of extensors, is greatly accelerated by sound in the contralateral ear. C. Inhibition of tonus by sound in the ipsilateral ear. Some recovery of tonus develops following cessation of the inhibitory stimulus.

In the presence of extensor hypertonus of the arm, sufficient to produce definite involuntary abduction of the limb, a sound of any frequency from 60 to 4096 cycles and of intensity greater than 30 decibels above the threshold of hearing will, when applied to the contralateral ear, augment the extensor tonus. When a sound of similar intensity is applied, by earphone, to the ipsilateral ear, extensor tonus is quickly abolished. Effects on flexor tonus, which are also easily demonstrable, are in the opposite direction with respect to the ear stimulated. Tonus changes in the leg correspond to those of the arm of the same side. Figure 1 presents tracings of typical photographic records of these effects.

Monaural studies with an audiometer indicate that thresholds are much higher for tones above 8000 cycles than for sounds of lower frequency. Binaural application of 60 cycle tones of approximately equal intensity demonstrates that the augmentory effects of contralateral stimulation may be suppressed by concurrent ipsilateral stimuli, but that the inhibition, in this case, develops much more slowly and incompletely than in the case when ipsilateral stimulation is used alone.

These acoustic reflexes may be combined successively or simultaneously with tonic neck, labyrinthine, optic and other reflexes previously described (1).

In the absence of a pre-existing state of tonus in the arm, acoustic stimulation of the opposite ear produces no definite development of tonus of the extensors. However, if the arm, which is initially hanging limp at the side, is either voluntarily or passively lifted and allowed to drop again to the side, a sufficient stretch reflex may be developed to serve as a "handle" for the acoustic augmentor effect to become manifest. Under these conditions reflex abduction of 5° to 10° may be effected.

Discussion. The significance of these acoustic reflexes is not entirely clear. It is evident that they fit into the general pattern of attitudinal and postural reflexes, but one can only speculate as to their importance in normal human subjects. Acoustic reflexes have been studied in decerebrate animals (2, 3, 4), and it has been pointed out that reactions to sound may fall in two categories: *a*, fear reactions elicited by intense or sudden noises, and *b*, orientation reactions concerned with turning of the ears and head and body toward the source of the sound. The reactions which we have studied seem to belong in this second group.

SUMMARY

Acoustic stimuli are shown to alter the post-contraction hypertonus of human limb muscles. Monaural stimulation results in augmentation of contralateral, and inhibition of ipsilateral, extensor tonus of arm or leg. Flexor hypertonus is affected in the opposite direction.

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SODIUM ION MOVEMENT BETWEEN THE INTESTINAL LUMEN AND THE BLOOD[1]

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Received for publication February 17, 1944

The absolute rate of movement of ions across the intestinal epithelium cannot be studied without resort to tracer methods, except in the case of ions which are not normally present in the blood. In the case of important constituents of the animal body tracer methods offer the only opportunity for measurement of actual rates of movement in the two directions: 1, Blood to gut, and 2, gut to blood. Net movement of any such material as the sodium ion is obviously not a measure of total movement in either direction except in the cases in which movement in the opposite direction is zero.

It is the purpose of this paper to describe experiments on sodium ion movement in which Na^{24} has been introduced, in some cases into the intestinal lumen, and in others into the blood, in a group of surgically prepared intestinal loop dogs, studied repeatedly in the unanesthetized state. Previous studies on transport of radiosodium across the intestinal epithelium (1, 2, 3, 4) have not distinguished between different levels of the intestine, nor have they been concerned with comparative rates of movement in both directions. They have confirmed the known fact that sodium ion is readily absorbed from the intestine and have shown how quickly it is distributed throughout the body. The latter point is important but does not bear directly on the subject of this paper. Studies (5) on absorption of radiosodium from gastric pouches have a more direct bearing and indicate characteristic differences in permeability between antral and stomach body mucosa, the former showing the greater.

The observations to be reported at this time will show that under the conditions employed sodium ion is moving both into and out of the gut at various levels, even while rapid net movement out is occurring; that there is a descending aboral gradient in permeability; that absolute rates of movement of sodium ion can be calculated, and that they are related to net absorption rates in the case of a particular segment level, further, that the several levels show characteristic differences in this relation; that sodium ion movements both out of and into the gut are dependent upon gut sodium concentration; and that the total sodium turnover in the intestine is calculable and large. Moreover it will appear that there is reasonably good quantitative agreement between the measured absolute rate of sodium movement in either direction and the calculated rate derived from measurements of the absolute rate in the opposite direction and the net transport rate.

[1] Aided by grants from the Rockefeller Foundation and the Graduate School of the University of Minnesota.

1. Experimental methods. *a. Surgical procedures and conduct of experiments.* Chronic Thiry-Vella loops of the jejunum and ileum between 30 to 40 cm. in length and Vella loops 15 to 20 cm. in length of the duodenum and colon were made in nine dogs. Animals were routinely fed a pound of raw hamburger the evening before the operation but were otherwise not fed anything but water for 24 hours prior to surgery. All surgical procedures were carried out with aseptic technique, under ether or nembutal anesthesia; and anastomoses were fashioned substantially according to the closed method of Martzloff and Burget (6). Duodenal loops in two dogs extended from just distal to the opening of the common bile duct to the ligament of Treitz, the proximal end being closed and the distal end brought out through a stab wound as a single stoma. The accessary pancreatic duct was transplanted into jejunum, after dissection (preserving the blood supply) had removed the pancreas and numerous minor ducts from the duodenum.

Jejunal loops in two dogs extended 30 to 40 cm. distal from the ligament of Treitz, and ileal loops in three dogs were constructed from the 30 to 40 cm. of gut proximal to the large intestine. In both instances the proximal and distal segments of the loops were brought out obliquely through stab wounds over the lower ribs, on the left in the case of the former, on the right in the latter. Following this procedure, simple compression against the thoracic cage readily avoids fluid leakage during tests. After ligature of the inferior mesenteric artery and vein at the level of its colic branches, and the adjacent vessels in the mesocolon, in a preliminary operation, collateral circulation developed sufficiently to permit an ileocolic anastomosis only a few centimeters from the rectal sphincter. In this fashion a loop of about two-thirds of the colon in two dogs was made by inverting the terminal ileum and exteriorizing the distal segment as a Vella fistula.

All dogs were carefully trained to lie quietly on tables specially constructed with raised edges and a slight tilt to allow free drainage of wash solution. The "better" animals would lie quietly for over two hours without requiring any restraint or admonition. Before each test and frequently during training (to obviate effects of conditioning), the loop was washed with several hundred milliliters of normal saline solution at 36° to 39°C., introduced via a multiperforated no. 16 French soft rubber catheter, inserted well into the loops. When wash fluid returned relatively free of mucus and epithelial debris, irrigation was discontinued and the catheter was left in situ a minimum of 30 minutes before the absorption experiment was begun. For each test 20.0 ml. of the solution to be studied was delivered from a volumetric pipette into the loop and after repeated mixings in the pipette chamber a 2.5 ml. sample was withdrawn and placed in a clean centrifuge tube. Enough air to empty it was then forced through the catheter and it was maintained thus, by a suitable clamp. Additional samples were collected each ten minutes as long as obtainable up to 30 minutes, care being taken each time to secure a representative mixed specimen, followed by air emptying of the catheter. At the conclusion of each absorption test 10 ml. of a 5 per cent sucrose solution was introduced from a volumetric pipette and

thoroughly mixed with the residual solution in the gut. An aliquot was then withdrawn and delivered into a clean dry centrifuge tube. The volume remaining in the loop was determined by comparing the conductivity of the last sample (0.2 ml.) diluted with 10 ml. (of water) and the sucrose wash solution (1 ml. diluted with 10 ml. of water) by means of a dipping conductivity cell. The electrodes were coated with platinum black and the cell was washed and dried after rinsing in alcohol and ether before each separate determination. The two solutions were kept at the same temperature in test tubes in a large beaker of water. It was assumed that the conductivity of the sucrose wash solution was directly proportional to the fraction of salt solution it had been mixed with in the gut. This method for determining volumes is very much quicker than analysis for a reference substance and has satisfactory accuracy. There is no suitable way to determine volumes in the loop at successive intervals before the last sample is removed so where necessary we have assumed that the change in volume was linear with time. This assumption is not valid over the whole of the absorption curve but, according to earlier observations (7), is substantially accurate until more than 50 per cent of the fluid has been absorbed. The possible importance of differences in length and surface area will be discussed later.

b. Analytical methods. Chloride was determined on 0.2 ml. samples according to Keys (8). One to 2 ml. samples were ashed with sulfuric acid at 500°C. for 5 hours or overnight. The ash was taken up to 1N HCl and an aliquot taken for potassium analysis according to Wilde (9). Sodium was determined on another aliquot by a micromodification of the standard zinc-uranyl-acetate method. All analytical methods were standardized by analyzing known solutions containing the same salts as the unknowns in the ranges of concentration employed.

c. Radiosodium technique. Na^{24} was prepared from pure sodium hydroxide by deuteron bombardment.[2] The hydroxide was converted to the chloride by addition of hydrochloric acid and brought to the desired concentration. As noted above, two types of experiments were performed, one in which the disappearance of Na* (labelled sodium) from the intestinal lumen was studied, and another in which the rate of entrance into the lumen from the blood was observed. In the first case the Na* solution was placed in the gut loop. In the second it was injected intravenously as an isotonic solution of NaCl 30 to 90 minutes before the start of the transport experiment. The radioactivity was measured by the use of Geiger counters of tested accuracy.[3] Background counts were made at appropriate intervals and decay rates were determined as a check on the methods and the identity of the radioactive material. In each case appropriate corrections for decay are made according to the standard procedure.

The radioactivity of the solutions is first determined on a volume basis. The value is referred to subsequently as RRA, that is "relative radioactivity", at a particular time. The quantities are subsequently expressed in two other ways,

[2] We are indebted to Prof. John H. Williams of the Department of Physics for supervising this procedure.

[3] We are indebted to Prof. Wallace D. Armstrong of the Division of Physiological Chemistry for the loan of apparatus and for advice in the radioactivity measurements.

"specific sodium activity" and "labelled sodium concentration". The specific sodium activity is defined in this paper as the radioactivity per unit of sodium concentration referred (a), in the case of the gut to blood experiments, to the radioactivity of the sodium in the gut at zero time as unity, and (b) in the blood to gut experiments, to the radioactivity of the sodium in the plasma as unity.

Thus, for case (a):

$$(SSA_g)_t = \frac{(RRA_g)_t}{[\mathrm{Na}^+{}_g]_t} \div \frac{(RRA_g)_0}{[\mathrm{Na}^+{}_g]_0}.$$

And, for case (b):

$$(SSA_g)_t = \frac{(RRA_g)_t}{[\mathrm{Na}^+{}_g]_t} \div \frac{(RRA_p)}{[\mathrm{Na}^+{}_p]}.$$

The mean value over any time t will be indicated as $\overline{\mathrm{SSA}}_g$. The labelled sodium concentration will be defined in the next section.

2. Methods for calculation of rates of sodium ion movement from tracer data. *a. For observations in which labelled sodium is placed in gut loops.* From the observed data the "labelled sodium concentration",[4] at any time is calculated as follows:

$$[\mathrm{Na}^*{}_g]_t = \frac{(RRA_g)_t}{(RRA_g)_0} \cdot [\mathrm{Na}^+{}_g]_0 \tag{1}$$

At zero time labelled sodium must by definition equal total sodium concentration.

The rate of total sodium ion movement will be measured by the quantity of radiosodium moved per unit time divided by the proportion of sodium ions moving which are radioactive. The amount of radio sodium moved is measured by the decline in labelled sodium in the gut loop, and the proportion of the sodium consisting of the radioactive isotope is given in relative values by the specific sodium activity. Since the labelled sodium concentration is already referred to the initial total sodium concentration, the fractional change in SSA over time can be used to account for the abundance ratio change over time in calculating total sodium movement. Thus:

$$\mathrm{Na}_{\mathrm{out}} = \frac{([\mathrm{Na}^*{}_g]_0 V_0 - [\mathrm{Na}^*{}_g]_t V_t) \cdot (SSA_g)_0}{\overline{SSA}_g \cdot t} \tag{2}$$

It will be obvious that Na_{out} will be expressed as milliequivalents per unit time if concentrations are expressed in mE per liter and volumes in liters. It

[4] It should be noted that labelled sodium concentration is not equivalent to Na^{24} concentration. The atoms of Na^{24} make up a very small proportion of the total sodium, but on the assumption that the intestinal epithelium does not distinguish between the several sodium isotopes the changes in amounts of any isotope present can be used to determine what has happened to the other isotopes present initially with the one studied. This assumption naturally underlies the use of isotopes as tracers in any situation. To the extent that this assumption is invalid any such measurements are in error. However, no evidence exists that the several sodium isotopes are treated appreciably differently by living membranes.

represents total sodium ion movement in the direction gut to blood per unit time. Equation 2 assumes that volume and concentration changes vary linearly with time. A few tests of this assumption indicate that it is approximately correct during the early periods of absorption.

In any two compartment systems between which materials can move in both directions the difference between the rates of movement in the two directions must represent the net transport rate. For the purposes of this analysis the gut loop can be considered as one compartment and the rest of the animal body as a second compartment. Furthermore it seems legitimate to confine attention to the blood as the portion of the second compartment with which the intestinal loop actually exchanges materials. It will not always be legitimate to ignore the multi-compartment nature of the remainder of the animal body, but for the situation in question it seems likely that no error will be introduced. Thus for the case of sodium ion movement it can be seen to be true that:

$$\mathrm{Na}_{\mathrm{out}} - \mathrm{Na}_{\mathrm{into}} = \mathrm{Na}_{\mathrm{net}} \tag{3}$$

where $\mathrm{Na}_{\mathrm{out}}$ is the rate of movement of sodium ion from gut to blood, $\mathrm{Na}_{\mathrm{into}}$ the rate in the reverse direction, and $\mathrm{Na}_{\mathrm{net}}$ the rate of net transfer across the epithelium between blood and gut. $\mathrm{Na}_{\mathrm{net}}$ can have either positive or negative values, the former indicating absorption and the latter excretion[5].

In these calculations it is assumed that the concentration of labelled sodium on the blood side of the intestinal epithelium is zero or negligibly small. Thus no account is taken of the possible return movement of radiosodium from blood to gut. The fact that the intestinal loop compartment has about one-thousandth the volume of the total body appears to justify such an assumption for the present case. A further assumption is made, namely, that the epithelial membrane has negligible thickness or volume capacity. It is believed that for the present calculations this assumption introduces no important error.

b. For observations in which labelled sodium is introduced into the blood. From the observed data the labelled sodium concentrations in the gut solutions are calculated as follows:

$$[\mathrm{Na}^*{}_g]_t = \frac{(RRA_g)_t}{(RRA_p)_t} \cdot [\mathrm{Na}^+{}_p]_t \tag{4}$$

where Na^+_p is the sodium concentration in the plasma in milliequivalents per liter.

The apparent rate of sodium ion movement from blood to gut is:

$$\mathrm{Na}_{\mathrm{into}}(\mathrm{uncorr.}) = \frac{([\mathrm{Na}^*{}_g]_t \cdot V_t) - ([\mathrm{Na}^*{}_g]_0 \cdot V_0)}{t} \tag{5}$$

This apparent rate is in error to the extent that labelled sodium moves from gut to blood during the time of observation. The situation is entirely different from that obtaining when the radiosodium is placed in the gut because in that

[5] The term excretion is used without any implication as to mechanism. The process may be entirely passive as far as the epithelium is concerned.

case the outside compartment is for all practical purposes infinitely large. In the case in question now, however, the labelled sodium in the gut compartment soon reaches finite values and to ignore return movement would vitiate any calculations based on observations over finite times. Therefore full account must be taken of this back movement. It can be accounted for in the following manner.

The true rate of movement of labelled sodium into the gut will be measured by the amount of labelled sodium accumulating in the loop per unit time plus the amount leaving in the same time, thus:

$$Na_{into} = Na_{into}\ (\text{uncorr.}) + X \tag{6}$$

where X equals the rate of loss of labelled sodium from the gut during the time of observation. The value of X can be ascertained to be:

$$X = Na_{out} \cdot \overline{SSA}_g \tag{7}$$

since the rate of loss of sodium entering from the blood must be equal to the rate of total sodium movement from gut to blood, times the mean specific radioactivity of the sodium in the gut. Equation 3, $Na_{out} - Na_{into} = Na_{net}$, applies by definition to experiments in which movement of labelled materials is in either direction. Substituting in equation 3 one obtains:

$$\frac{X}{\overline{SSA}_g} - [Na_{into}(\text{uncorr.}) + X] = Na_{net} \tag{8}$$

Rearranging and solving for X

$$X = \frac{[Na_{net} + Na_{into}(\text{uncorr.})] \cdot \overline{SSA}_g}{1 - \overline{SSA}_g} \tag{9}$$

In this equation X is the only quantity for which observational measurements are not available. It thus becomes possible to calculate X and apply the desired correction in equation 6. Likewise it is possible to calculate Na_{out} from either equation 7 or 3. The results of such calculations must be identical for mathematical reasons.

A more rigid and detailed treatment of the theoretical aspects of the calculation procedure has been made with the help of Mr. John M. Reiner. This treatment will be published in the near future in connection with the further mathematical analysis of the results of these and other experiments. The important clarification arising from the more rigid mathematical analysis is in showing that the deviation from linearity in volume and concentration changes observed is not great enough to alter any of the calculations presented here significantly.

3. Observations on concentration and specific radioactivity changes. *a. Simultaneous movement in two directions.* In figure 1a one sees the analytical results of an experiment in which Na^{24} in an isotonic solution of $MgSO_4$ and NaCl, each salt contributing about half of the total osmotic activity, was placed in a colonic loop and transport studied. It will be noted that both sodium and

chloride concentrations are falling, indicating the movement of those ions counter to their concentration gradients, since each ion is in higher concentration in the blood plasma than in the gut loop. This phenomenon has been the subject of earlier study (10). It will be noted, moreover, that the labelled sodium concentration in the intestine $[Na^*_g]$ diminishes very much more rapidly than does the sodium concentration $[Na^+_g]$, indicating that the absolute rate of sodium

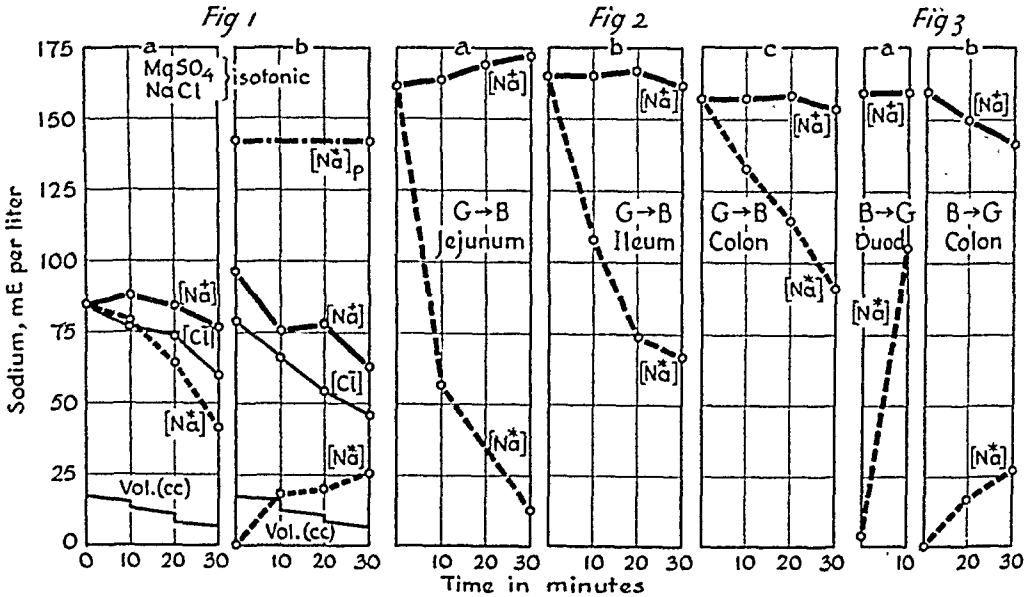

Fig. 1 a. Observations on changes in composition of fluid in a segment of colon in a dog in which the sodium was labelled by addition of Na^{24}. The concentration of labelled sodium [Na*] is expressed as indicated in the text. The solution in the gut was an isotonic mixture of equi-osmotic proportions of NaCl and $MgSO_4$.

Fig. 1 b. Observations on the same dog used in the experiment in figure 1a, in which Na^{24} was injected in isotonic NaCl into the blood 30 minutes before the start of the experiment. The movement of labelled sodium into the gut is to be noted. The gut solution was an isotonic mixture of equi-osmotic proportions of NaCl and $MgSO_4$.

Fig. 2 a, b and c. Changes in composition of isotonic NaCl solutions containing Na^{24} in dogs with surgically prepared isolated segments of jejunum, ileum and colon. The broken lines indicate concentrations of labelled sodium. Labelled sodium is moving from gut lumen to blood.

Fig. 3 a and b. Changes in composition of isotonic NaCl solutions in duodenal and colonic bowel segments in dogs injected intravenously with Na^{24} in isotonic NaCl 30 minutes or more before the beginning of observations. The broken line indicates labelled sodium concentration.

movement will be found to be much greater than would be suggested by the sodium concentration change. Calculations to be presented in section 4 will confirm this deduction.

Such an observation would indicate that even while there is a large net movement of sodium from gut to blood ($G \rightarrow B$) there must be a considerable rate of movement in the opposite direction, that is from blood to gut ($B \rightarrow G$). In order to obtain direct proof of such a back movement it is necessary to study labelled sodium movement from $B \rightarrow G$ while the net movement is, as before,

in the opposite direction. The results of a typical experiment of this sort are presented in figure 1b. It can be seen that there is a large movement of labelled sodium from $B \rightarrow G$, even while the net sodium movement is large in the opposite direction. This has been the invariable result in all comparable experiments. It indicates that net movement of sodium will be found to be the algebraic sum of movements in the two directions: gut to blood, and blood to gut; and that the back movements can be very large.

b. Observations on the behavior of the intestine at various levels. The several segments of the intestine possess differing properties with respect to the transport of materials between lumen and blood. It is obviously of interest to know about the relative freedom of movement of ions in the directions $G \rightarrow B$ and $B \rightarrow G$ at various levels from duodenum to colon. Figure 2a, b and c, and figure 3a and b, present data on concentration changes when there is movement of labelled sodium from and into isotonic NaCl solutions at four levels. Figure 2, in its three sections, shows the changes in $[Na^+_g]$ and $[Na^*_g]$ over time when the radiosodium is placed in the gut loop. Each figure is the average of two or more experiments, the entire number performed under a given condition. The striking fact to be noted is the slower rate of change in labelled sodium concentration as one proceeds to the aboral end of the gut. The rate of fall in $[Na^*_g]$ in the jejunum is about five times that in the colon. Figure 3 presents the observations for the reverse situation, $B \rightarrow G$, in the cases of the duodenum and colon. Here it is to be noted that the rate of change in $[Na^*_g]$ is about ten times as great in the duodenum as in the colon. It therefore appears that the freedom of movement and the rate of movement of sodium ion between the blood and isotonic NaCl in the intestinal lumen are less, in either direction, as one proceeds to the aboral end of the gut.

Results of studies on movement of labelled sodium out of and into isotonic Na_2SO_4 solutions are shown in figures 4a, b and c, and 5a, b and c. Again it is to be seen that rates of change in $[Na^*_g]$ are smallest in the case of the colon, largest for the jejunum and intermediate for the ileum. Thus the aboral gradient is again indicated. The differences between colon and jejunum are between four and six fold. A minor point in connection with figure 5a should be noted. At 30 minutes $[Na^*_g]$ exceeds $[Na^+_g]$ by about 10 per cent. This relation might be accounted for either on the grounds of experimental error or on the basis of a fall in the specific radioactivity of sodium in the blood over time.

c. The general relation between sodium ion concentration and movement between blood and gut. The rate at which radiosodium is cleared from the gut has been studied in experiments in which the total sodium ion concentration in the gut solutions was set at various levels from 20 to 220 mE per liter. The converse rate of entrance into the gut has been studied under comparable conditions. Figure 6a, b and c show the average results of experiments under the conditions in question upon the colon, studying movement in the direction $G \rightarrow B$. It can be seen that the absolute rate of change in $[Na^*_g]$ varies directly with the sodium concentration. This indicates that the rate of movement of labelled sodium from gut to blood will be found (see p. 500) to be greater the higher the

sodium concentration. Such a relation is perhaps to be expected. Likewise the rate of increase in $[Na^*_g]$ in the colon loop is seen (fig. 7a, b and c) to be greater the higher the sodium concentration. The significance of this observation will become more evident later when calculations of rates of movement are presented.

The establishment of equilibrium between blood and gut with respect to the specific activity of sodium is, as might be expected, faster the lower the sodium content of the gut solution. This is evident from the fact, as seen in figure 7a, b and c, that $[Na^*_g]$ approaches $[Na^+_g]$ more completely at any given time, the

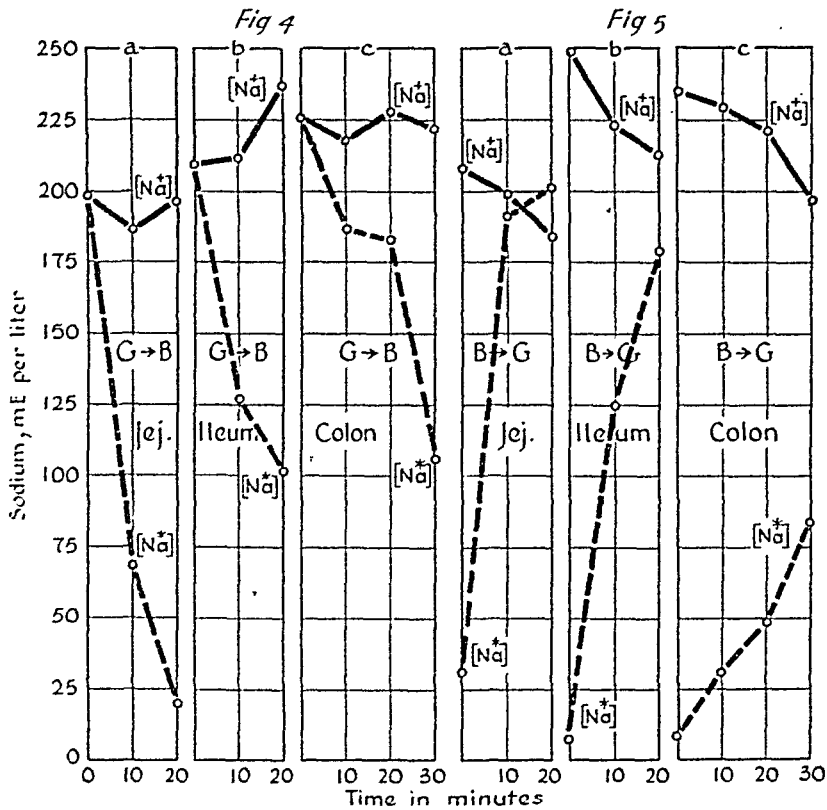

Fig. 4 a, b and c. Changes in composition of isotonic Na_2SO_4 solutions with sodium labelled in jejunal, ileal and colonic segments. The movement of Na^{24} is from gut to blood.

Fig. 5 a, b and c. Changes in composition of Na_2SO_4 solutions as in figure 4, and in the same dogs, but in which the blood sodium was labelled. The movement of Na^{24} is from blood to gut.

lower the value of $[Na^+_g]$. This relation can be seen to hold at all levels of the gut studied in tables 1 and 2 by inspection of the values for specific radioactivity of sodium in the gut loops at 10 minutes after insertion of samples. In table 1 data appear for experiments in which Na^{24} was placed in the gut. The trend is evident for the specific activity of sodium at 10 minutes to be lower, the lower the sodium concentration in the loop. In table 2 are the data for experiments in which Na^{24} was put in the blood. It will be noted on inspection that the specific activity of sodium at 10 minutes tends to be higher, the lower the sodium concentration; that is, in both cases, the degree of approach to equilibrium

between gut and blood with respect to the isotopic composition of the sodium in each is greater the lower the sodium content of the gut fluid.

4. CALCULATIONS OF ABSOLUTE RATES OF SODIUM MOVEMENT BETWEEN INTESTINAL LUMEN AND BLOOD. In fifty-seven experiments complete analytical data have been obtained for measurements of movement of sodium ion between blood and gut lumen for jejunum, ileum and colon.[6] These data and the calculations of rates of movement for the first 10 minutes of observation made from them are presented in tables 1 and 2. The values for rates of movement in

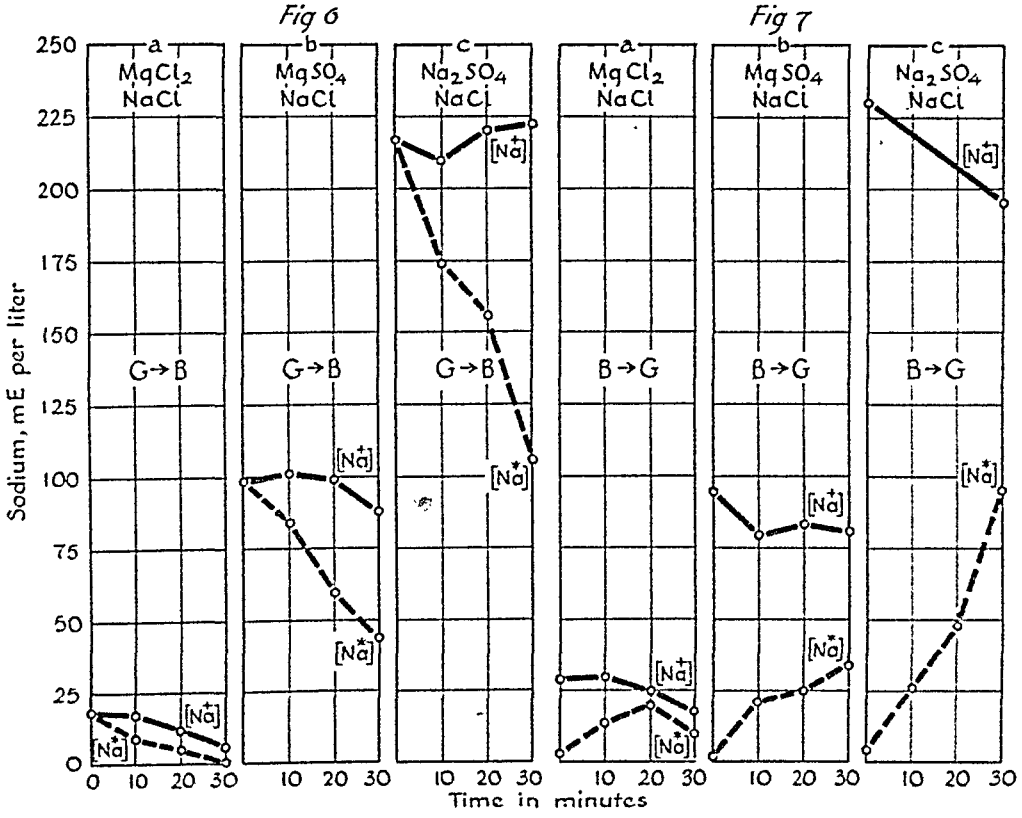

Fig. 6 a, b and c. Changes in composition of isotonic solutions of mixtures of the salts indicated, with varying proportions of NaCl as indicated by Na^+ at zero time in the colon. The movement of labelled sodium is from gut to blood.

Fig. 7 a, b and c. Changes in composition of isotonic solutions of mixtures of the salts indicated in the same colon segments used in the experiments of figure 6. The movement of labelled sodium is from blood to gut.

table 1 are calculated according to equations 2 and 3, and those in table 2 according to equations 6 and 3. A number of additional experiments were made but incompleteness of data prevents their being included in these tabulations. In view of the number of experimental situations employed it is difficult to analyze the data in these tables except in graphic form. Therefore the significant correlations are presented in a series of graphs.

[6] Relatively few observations were made on the duodenum and they are therefore not presented at this time. These few observations indicate a behavior somewhat similar to the jejunum.

The first point of importance is to ascertain the correlation between the concentration of sodium ion and the rates of movement of sodium out of and into

TABLE 1

Analytical data and calculations for experiments in which labelled sodium moved from gut to blood

LEVEL OF INTESTINE AND CHARACTER OF SOLUTION IN APPROXIMATE FRACTIONS OF ISOTONICITY			V_0	V_{10}	$[Na^{22}]_0$	$[Na^{22}]_{10}$	Na^{24} SSA_{10}	Na_{out}	Na_{into}	Na_{net}
			cc.	*cc.*	*mE/l*	*mE/l*		*mE/min.*	*mE/min.*	*mE/min.*
Jejunum										
Expt. 1	0.5 NaCl	0.5 Na_2SO_4	17.4	14.4	214	205	0.272	0.459	0.382	0.077
2	0.5 NaCl	0.5 Na_2SO_4	17.5	19.8	190	193	0.315	0.323	0.373	−0.050
3	1.0 NaCl		17.5	14.8	162	172	0.490	0.214	0.184	0.030
4	0.5 NaCl	0.5 $MgSO_4$	17.5	17.2	76	98	0.123	0.200	0.235	0.035
5	0.5 NaCl	0.5 $MgSO_4$	17.4	20.4	72	110	0.154	0.157	0.256	−0.099
6	0.4 NaCl	0.6 $MgCl_2$	17.6	14.1	67	67	0.194	0.167	0.143	0.024
7	0.4 NaCl	0.6 $MgCl_2$	17.8	13.6	64	86	0.070	0.198	0.200	−0.002
Ileum										
8	0.1 NaCl	0.9 Na_2SO_4	16.7	14.1	251	247	0.810	0.151	0.081	0.070
9	0.1 NaCl	0.9 Na_2SO_4	16.5	13.1	242	240	0.345	0.431	0.346	0.085
10	0.5 NaCl	0.5 Na_2SO_4	17.6	15.9	241	240	0.603	0.242	0.200	0.042
11	0.5 NaCl	0.5 Na_2SO_4	17.4	14.9	230	225	0.532	0.290	0.226	0.064
12	0.5 NaCl	0.5 Na_2SO_4	17.5	15.5	196	209	0.657	0.157	0.138	0.019
13	1.0 NaCl		17.4	11.2	169	179	0.491	0.263	0.168	0.095
14	1.0 NaCl		17.5	14.5	165	158	0.887	0.090	0.031	0.059
15	1.0 NaCl		17.3	14.1	162	158	0.604	0.182	0.124	0.058
16	0.5 NaCl	0.5 $MgSO_4$	17.5	14.6	81	96	0.431	0.113	0.112	0.001
17	0.5 NaCl	0.5 $MgSO_4$	17.5	15.6	74	82	0.598	0.066	0.065	0.001
18	0.5 NaCl	0.5 $MgSO_4$	17.4	16.2	77	95	0.543	0.064	0.084	−0.020
19	0.1 NaCl	0.9 $MgSO_4$	16.8	16.1	20	49	0.099	0.045	0.090	−0.045
20	0.1 NaCl	0.9 $MgSO_4$	16.5	14.3	24	46	0.283	0.033	0.059	−0.026
21	0.1 NaCl	0.9 $MgCl_2$	16.5	12.0	21	18	0.309	0.042	0.029	0.013
22	0.1 NaCl	0.9 $MgCl_2$	16.4	14.0	19	31	0.417	0.018	0.031	−0.013
23	0.1 NaCl	0.9 $MgCl_2$	17.6	13.2	18	22	0.195	0.043	0.041	0.002
24	0.1 NaCl	0.9 $MgCl_2$	17.7	15.1	17	26	0.335	0.025	0.034	−0.009
Colon										
25	0.1 NaCl	0.9 Na_2SO_4	16.5	13.0	238	243	0.885	0.121	0.044	0.077
26	0.5 NaCl	0.5 Na_2SO_4	17.3	15.7	230	210	0.919	0.099	0.031	0.068
27	1.0 NaCl		17.9	14.5	162	156	0.868	0.104	0.040	0.064
28	1.0 NaCl		17.5	15.4	152	158	0.827	0.071	0.048	0.023
29	0.6 NaCl	0.4 $MgSO_4$	17.6	14.5	110	113	0.769	0.076	0.046	0.030
30	0.5 NaCl	0.5 $MgSO_4$	17.5	15.5	85	88	0.871	0.034	0.021	0.013
31	0.1 NaCl	0.9 $MgSO_4$	16.4	13.8	16	17	0.613	0.015	0.013	0.002
32	0.1 NaCl	0.9 $MgCl_2$	16.5	14.7	21	14	0.534	0.030	0.016	0.014
33	0.1 NaCl	0.9 $MgCl_2$	17.9	15.8	18	19	0.474	0.024	0.022	0.002

the gut at the several levels. The second point of major interest is to ascertain whether the rate of movement "observed" in one direction is in agreement with

the rate "calculated" from labelled sodium movement in the opposite direction; whether, in other words, the data obtained can be employed in the equation:

$$Na_{out} - Na_{into} = Na_{net}$$

A third correlation of interest is that between the movement out of and into the gut. A fourth correlation to be studied is the relation between absolute rates of movement in either direction and the net transport rate.

TABLE 2

Analytical data and calculations for experiments in which labelled sodium moved from blood to gut

LEVEL OF INTESTINE AND CHARACTER OF SOLUTION IN APPROXIMATE FRACTIONS OF ISOTONICITY	V_0	V_{10}	$[Na^{22}]_0$	$[Na^{22}]_{10}$	Na^{24} SSA$_0$	Na^{24} SSA$_{10}$	Na_{out}	Na_{into}	Na_{net}
	cc.	*cc.*	*mE/l*	*mE/l*			*mE/min.*	*mE/min.*	*mE/min.*
Jejunum									
Expt. 1 0.5 NaCl 0.5 Na_2SO_4	17.5	17.0	217	198	0.147	0.880	0.580	0.538	0.042
2 0.5 NaCl 0.5 Na_2SO_4	17.4	14.7	200	200	0.145	1.00	0.754	0.700	0.054
3 0.6 NaCl 0.4 $MgCl_2$	17.5	15.5	102	91	0.056	0.846	0.266	0.229	0.037
4 0.03 NaCl 0.97 $MgCl_2$	17.5	15.5	5	26	1.00	0.924	0.120	0.152	−0.032
Ileum									
5 1.0 Na_2SO_4.....	17.3	11.7	312	218	0.000	0.720	0.733	0.448	0.285
6 0.4 NaCl 0.6 Na_2SO_4	17.5	15.8	238	228	0.023	0.391	0.237	0.181	0.056
7 0.5 NaCl 0.5 Na_2SO_4	17.4	12.9	230	200	0.008	0.526	0.374	0.232	0.142
8 0.5 NaCl 0.5 Na_2SO_4	17.5	14.8	230	218	0.000	0.303	0.209	0.129	0.080
9 0.5 NaCl 0.5 Na_2SO_4	17.5	14.5	230	220	0.013	0.400	0.259	0.176	0.083
10 0.6 NaCl 0.4 $MgSO_4$	17.5	14.8	93	100	0.000	0.600	0.148	0.133	0.015
11 0.5 NaCl 0.5 $MgSO_4$	17.5	16.2	89	99	0.045	0.414	0.072	0.076	−0.004
12 0.5 NaCl 0.5 $MgSO_4$	17.5	15.8	80	90	0.000	0.378	0.068	0.065	0.003
13 0.5 NaCl 0.5 $MgSO_4$	17.3	13.5	84	85	0.060	0.493	0.109	0.078	0.031
14 0.3 NaCl 0.7 $MgCl_2$	17.4	13.7	40	61	0.000	0.852	0.100	0.114	−0.014
15 0.3 NaCl 0.7 $MgCl_2$	17.4	14.5	34	54	0.000	0.402	0.015	0.034	−0.019
16 1.0 $MgCl_2$.....	17.5	14.5	3	13	0.000	1.000	0.020	0.035	−0.015
Colon									
17 1.0 NaCl.....	17.5	13.8	160	150	0.000	0.113	0.103	0.029	0.074
18 0.5 NaCl 0.5 Na_2SO_4	17.6	14.4	228	211	0.018	0.161	0.153	0.056	0.097
19 0.5 NaCl 0.5 Na_2SO_4	16.8	14.7	232	226	0.013	0.080	0.083	0.025	0.058
20 0.6 NaCl 0.4 $MgSO_4$	17.3	15.5	96	76	0.000	0.237	0.086	0.038	0.048
21 0.6 NaCl 0.4 $MgSO_4$	17.3	16.3	94	95	0.075	0.253	0.042	0.034	0.008
22 0.5 NaCl 0.5 $MgSO_4$	15.3	13.8	90	69	0.000	0.116	0.057	0.014	0.043
23 0.4 NaCl 0.6 $MgCl_2$	17.5	15.2	54	51	0.100	0.381	0.049	0.032	0.017
24 1.0 $MgCl_2$.....	17.4	14.7	3	9	0.000	1.000	0.014	0.021	−0.007

In figure 8a are plotted the rates of sodium ion movement from gut to blood at the levels of the jejunum, ileum and colon, at the several concentrations of sodium ion in the gut fluid, obtained from the experiments in which radiosodium was placed in the gut and its loss to the blood observed. In these studies the rate Na_{out} is the "observed" rate corrected from the gross measurements, as

indicated in equation 4, by taking the decline in specific radioactivity of sodium over the time of observation into account.

Figure 8b presents the results of calculation of the same rate, Na_{out}, derived from the observations in which labelled sodium moved into the gut. In this case Na_{into} and Na_{net} were measured and Na_{out} calculated from them. As has been noted, the rate Na_{into} is calculated by taking back movement into account.

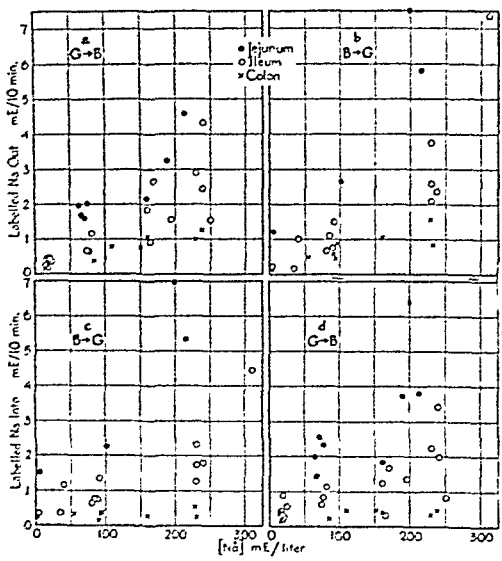

Fig. 8

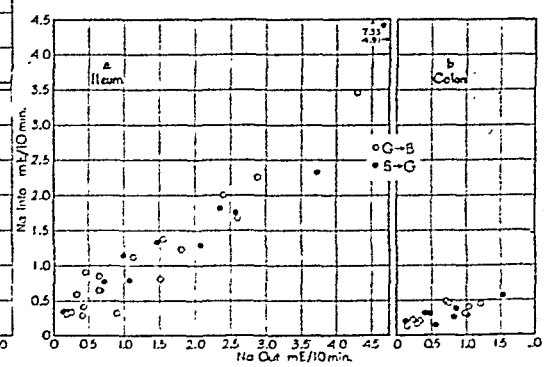

Fig. 9

Fig. 8 a. Rates of movement of sodium *out* of the gut plotted against gut sodium concentration for loops at three levels of intestine, measured in experiments in which radiosodium moved from *gut to blood*.

Fig. 8 b. Rates of movement of sodium *out* of the gut calculated from experiments in which radiosodium moved from *blood to gut*.

Fig. 8 c. Rates of movement of sodium *into* the gut measured in experiments in which radiosodium moved from *blood to gut*.

Fig. 8 d. Rates of movement of sodium *into* the gut calculated from experiments in which radiosodium moved from *gut to blood*.

Fig. 9 a. The relationship between the values obtained for the rates Na_{into} and Na_{out} in the case of the ileum. The open circles represent values obtained when radiosodium was placed in the gut and the measured total movement was Na_{out}, the value for Na_{into} being calculated. The solid dots are values obtained when radiosodium was put in the blood and Na_{into} measured, while the value for Na_{out} was calculated as indicated in the text.

Fig. 9 b. The same type of data from observations upon the colon.

Inspection of these two figures shows, first, that there is a rather high degree of variability in rates of sodium movement from each of the three levels of gut, which is least in the colon. This variability is not so great, however, as to obscure certain facts, namely, that there is a positive correlation between Na_{out} and $[Na^+_g]$, that the rates for Na_{out} are characteristically different for the segments at each gut level, and that the general ranges of values are the same in the two graphs. Especially in the case of the colon there is no doubt that the "observed" and "calculated" rates for Na_{out} at equal sodium concentrations are on the average very nearly the same in the seventeen observations which have been made.

In the case of the ileum, the scatter of points in the two graphs covers the same general area of the rate-concentration plot. In connection with this rather wide scatter in the cases of the ileum and jejunum it is believed that the variations noted are largely real and not due in any great part to errors in measurement. It must be admitted that since six quantitative measurements are represented in each point there is a possibility of accidental summation of random errors which might result in a large final error. There is, however, no evident reason why such random errors in measurement should be greater in the ileum or jejunum than in the colon. Therefore it appears that the variability of behavior of the ileum and jejunum are real, and result from a greater sensitivity to factors which it was not possible to control in these experiments. It seems very likely that important information would be gained if it were possible to ascertain the causes of the observed variations.

It should be noted that the rate Na_{out} becomes very small at low values for $[Na^+_g]$. If straight lines describing the means of observed values be drawn for each, the ileum and the colon, it will be noted that when extended to zero $[Na^+_g]$ they would fall at 0.25 mE per 10 min. or below. It is of interest that no quantity calculated for Na_{out} from B→G experiments had a negative sign. This would be entirely possible if random errors were large. The absence of such a finding speaks for the view expressed above that the observed day to day variability in the jejunum and ileum is real.

The observed values for the reverse rate, that of Na_{into} the gut, are plotted against concentration in figure 8 c, and the calculated values, from experiments in which radiosodium moved from gut to blood, in figure 8 d. It is to be noted first that the grouping of values for each of the three gut levels is in the same order as before. Next, it is possible to see that in the case of the ileum, and more obviously so in the case of the colon, the average values for Na_{into} at any of the higher gut sodium concentrations are much smaller than the values for Na_{out} shown in figures 8 a and b. It seems significant that this is true for the "calculated" values shown in figure 8 d. Calling especial attention to the colon values, because their variability is least, it will be seen that the agreement between "observed" and "calculated" values for Na_{into} is fairly good. In the case of the ileum the situation is exactly as was seen in connection with the comparison of the two sets of values for Na_{out}. The scatter of values for "observed" and "calculated" Na_{into} rates covers the same area.

The relation between the rates of movement into and out of the intestine can be seen better when these two variables are plotted one against the other. If the rates Na_{into} and Na_{out} were equal the points representing each experiment should fall on the 45° line of the graph. The deviation from 45° will thus be a measure of the difference between the two rates in a given case. By plotting the results of both types of experiment, namely, the G→B and B→G experiments, on the same graph, it becomes possible to ascertain whether the two methods of measurement lead to reciprocally corresponding values. Figures 9 a and b present such figures for the ileum and colon respectively. It is evident from these graphs that there is a marked difference between the two levels of gut,

the colon possessing regularly a lower rate for Na_{into} than is characteristic of the ileum. This difference is largest at the higher rates of movement.

The second feature to be noted in figure 9 is that for both levels of gut the values obtained in the B→G and in the G→B experiments fall in the same areas of the graphs. This fact constitutes further confirmation of the general validity of the methods of calculation from movements in the two directions.

The fourth important relationship which can be ascertained from the observed data is that between net sodium movement and the two directional movements. These relations are shown in figures 10 a, b, c and d. In the first two, Na_{net} is plotted against Na_{out}, in the last two against Na_{into}. It will be evident upon

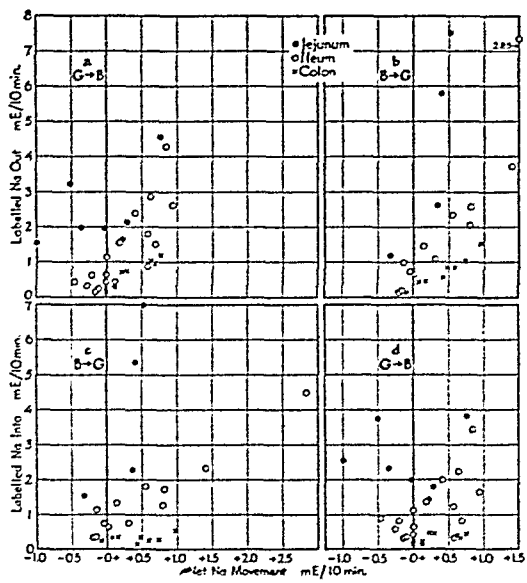

Fig. 10 a. The relation between *total* movement of sodium *out* of the gut, at three levels, and the *net* sodium movement observed in experiments in which radiosodium moved from *gut to blood*.

Fig. 10 b. The same relation as shown in figure 10 a, derived from experiments in which the radiosodium moved from *blood to gut*.

Fig. 10 c. The relation between *total* movement of sodium *into* gut and the *net* sodium movement observed in experiments in which radiosodium moved from *blood to gut*.

Fig. 10 d. The same relation as shown in figure 10 c, derived from experiments in which radiosodium moved from *gut to blood*.

inspection that the three levels of intestine behave very differently with respect to the relation shown in these graphs. The jejunum shows very low, or negative, values of Na_{net} for large values of Na_{out} or Na_{into}. Exactly the reverse is seen in the case of the colon. The ileum behaves in an intermediate fashion.

5. Discussion of results. The data and calculations which have been presented provide quantitative measurements of sodium ion movement in both directions between intestinal lumen and blood. It has been shown that under all conditions studied there is movement of sodium ion in both directions whenever there is movement in either. The magnitude of movement from blood to gut calculated or observed is less than the reverse movement under all conditions

in which there is net sodium absorption. This is true regardless of whether the sodium concentration in gut fluid is higher or lower than that in the plasma.

The movement of sodium from blood to gut is greater the higher the gut sodium concentration under the conditions studied in the jejunum and ileum. In the colon it appears that this situation obtains if at all only at the lower concentration levels. The return movement of material undergoing absorption represents a "leak" in one sense. The most efficient absorbing mechanism would appear to be one in which movement is strictly unidirectional. However, absorption is not the only function of the intestine and hence it would be improper to expect to find a mechanism which ignored other requirements. It may be of importance that the colon approaches the condition of unidirectional movement to a greater extent than does either the ileum or jejunum. In the case of the colon, where absorptive functions predominate, the rate of sodium movement into the gut at the higher sodium concentrations is less than 50 per cent as great as the reverse rate. The jejunum is obviously unfitted for absorption of sodium against concentration gradients, the colon possesses a mechanism beautifully adapted to such a function, and the ileum is able under optimum conditions to accomplish positive net movement nearly as well as the colon, but in the average case is definitely less efficient.

The difference in length of intestinal segment employed at the several levels of the intestine constitutes a complicating feature of these experiments. As noted under methods, the duodenal and colonic loops were made approximately one-half the length of the other two types. A correction for this difference will only result in an accentuation of the greater apparent permeability found for the duodenum, but if a linear correction is made it would reduce the difference between ileum and colon. In fact as between these two it would leave a significant difference only in the case of movement from blood to gut. It would be necessary to know the surface area available for permeation at each level of intestine in order to make further deductions. No such area measurements were attempted.

It should also be noted that in both the ileum and the colon, but more particularly in the latter, the rate Na_{out} exceeds Na_{into}, even when the $[Na^+_g]$ is very much smaller than $[Na^+_p]$. This result would, of course, have been predicted from the fact that Na_{net} is positive under these conditions. Nevertheless it is significant that direct observation of labelled sodium movement in the B→G experiments yields the result anticipated. This agreement with expectation gives support to the view that the method of calculation of rates of movement which has been employed is essentially correct. Its validity is strengthened further by the fact that there is general agreement between the observed and calculated values for rates of movement in the two sets of experiments in which labelled sodium moved in one case into and in the other out of the gut.

An approximate calculation of the total amounts of sodium moved into and out of the gut under various conditions can be made from the data herewith presented. The lengths of the jejunum, ileum and colon of a 10 kgm. dog may be taken as about 70, 70 and 25 cm. respectively. The segments employed

in these studies were each approximately one half of the jejunum and ileum and two thirds of the colon. The rates for Na_{into} the several gut segments at 160 mE $[Na^+_g]$ were found to be about 0.25, 0.14 and 0.04 mE per min. respectively. The calculated rate of entrance of sodium into isotonic NaCl for the entire intestine, exclusive of duodenum, is calculated to be 0.84 mE per min. Assuming the dog's plasma volume to be 5 per cent of its weight and the $[Na^+_g]$ to be 140 mE/l., there would be 70 mE of sodium in the plasma. An amount equal to this would pass into the gut in 83 minutes in the case in question. This calculation is obviously rough, and a more refined one is at present unjustified but it will serve to show the relatively large magnitudes of transport rates of material into and out of the gut. If the same general conditions occur in the human one might calculate that for a man weighing 60 kgm. the rate of turnover would be about 5.0 mE per min. or 162 grams per day, which is twenty-five or more times the usual daily sodium intake. Since the lumen of the gut normally contains roughly the concentration of sodium assumed in this calculation it seems likely that a turnover of this general order of magnitude actually occurs. This is in addition to any sodium secreted by the various digestive glands in response to food. These calculations have been made primarily to indicate the large quantities involved, and to point out that a defect in the absorbing mechanism need not approach the stage of total breakdown of function in order to result in drastic salt loss from the body.

In the calculations of movement between gut lumen and blood which have been presented the mechanisms of movement have intentionally been ignored. The proportionate parts played by the passive permeability characteristics of the intestinal epithelium and by active work processes have not been considered.

It has been shown in the studies reported here that the apparent permeability of the small gut to sodium ion entering from the blood is positively correlated with the rate at which the univalent cation present is leaving the gut. There is no positive proof that the relationship is causal, but it seems very likely to be. If so it would be concluded that the apparent permeability to movement in one direction would depend upon a dynamic process occurring in the opposite direction. Capacities for penetration by diffusion can be made evident in a dynamic system only when active movements are measurable and controlled. It thus becomes evident that the term permeability in a dynamic system has only a limited meaning. Structural factors alone will not account for the observed facts. The degree to which structural characteristics can control permeation depends upon dynamic factors. In another communication data on simultaneous studies of water and inorganic ion movement by tracer methods will be presented and a fuller discussion will be given at that time.

SUMMARY

a. Na^{24} has been employed as a tracer in measuring rates of sodium ion movement into and out of the gut at several levels in chronic surgically prepared segments of the dog intestine. Methods of observation and calculation have been described.

b. The absolute rates of movement of material in either direction can be calculated, knowing the net transport rate and the absolute movement rate in one direction. Rates determined from measurements of net transport and of total movement in either direction show general agreement under comparable conditions.

c. Sodium ion moves in both directions across the intestinal epithelium at measurable rates under all conditions studied. Even when there is movement of sodium from gut to blood against large concentration gradients small amounts of that ion are simultaneously entering the gut from the blood.

d. There is in general a descending aboral gradient in sodium movement from blood to gut, the movement being least in the colon.

e. The same general rule holds for movement in the gut to blood direction except that there is probably little difference between ileum and colon.

f. The rates of sodium ion movement both out of and into the gut are both positively correlated with sodium ion concentration in the small gut under the conditions of the experiments reported. This correlation could not have been predicted for movement into the gut and probably has significance in connection with the dynamics of movement of sodium ion.

g. The jejunum and ileum show greater variability in measured rates of sodium ion movement than does the colon. It is believed that the variations observed in the first two regions result from real changes in state of the animal from day to day. The causes of the variations were not ascertained.

h. The difference between Na_{out} and Na_{into} is ordinarily much greater for colon than for ileum. It appears therefore that the colon possesses a more efficient absorbing mechanism with a smaller "leak".

i. A rough calculation shows that the total turnover rate of sodium between the intestine and the blood is very large, being approximately equal to the total plasma sodium per 83 minutes.

j. These observations stress the importance of dynamic factors in determining the apparent permeability of living membranes. The fact that sodium ion movement in a given direction through a structure readily permeable to it is positively correlated with the simultaneous rate of the univalent cation movement through it in the opposite direction, indicates that permeation under these conditions depends upon dynamic factors more than upon structural ones.

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THE PEPSIN CONTENT OF GASTRIC JUICE SECRETED IN RESPONSE TO HORMONAL STIMULATION

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Received for publication March 30, 1944

Gregory and Ivy (1) have presented evidence indicating that the stimulation of secretion in a subcutaneously transplanted pouch by perfusion of a second pouch of the remainder of the stomach with secretagogues is mediated by a hormonal mechanism. Butler, Hands and Ivy (2) have confirmed and extended the report of Kim and Ivy (3) to the effect that gastric secretagogues are more potent and have a lower threshold dose when administered by local perfusion of the gastric mucosa than when administered slowly intravenously. This indicates that secretagogues do not act solely by absorption into the blood and that they probably cause the release of a hormonal gastric secretory agent.

Lim, Loo and Liu (4) observed that pepsin concentration is not always increased and not infrequently may be lowered in the gastric juice secreted in response to a meal by an autotransplanted pouch. We (5) have shown that the peptic secretory response of the transplanted gastric pouch to pharmacologic stimulants is similar to that of ordinary innervated and denervated gastric pouches.

This investigation is concerned with a study of the peptic secretory response of the transplanted pouch in response to the hormonal type of stimulus referred to above as compared with the response to the parenteral administration of histamine and pilocarpine.

METHODS. *Animals.* Two female dogs with large subcutaneously transplanted pouches of one-third of the fundic portion of the stomach and vagally denervated pouches of the remainder of the stomach served as experimental animals. The operative procedure is described in detail in the paper by Gregory and Ivy (1).

Stimulation of secretion and collection of samples. Histamine, pilocarpine and histamine-free liver extract (Lederle, 15 APA units per cc.) were used as secretory stimuli. Gastric juice was collected only from the transplanted pouches. Four samples were collected at half-hour intervals after the first drop of juice appeared in the collecting bottle. Histamine dihydrochloride was administered subcutaneously every 10 minutes in small doses calculated to give a volume of secretion approximately equal to that elicited by liver extract stimulation. The dosage of histamine dihydrochloride varied between 0.025 and 0.05 mgm. per 10 minutes. Three-tenths of a milligram of pilocarpine hydrochloride was administered subcutaneously every ten minutes. The liver extract was perfused through the main gastric pouch by a technique similar to that used by Butler et al. (2).

Determination of pepsin. Pepsin was estimated by the method of Anson (6) after appropriate dilution of the sample of gastric juice with 0.01 N HCl.

RESULTS. Samples were tested for free acid with alkacid paper and all were found to be below pH 3.0. The pepsin concentrations during the course of the two hour period showed no consistent or marked fluctuations, therefore only the total values for the two hour period are listed (table 1).

DISCUSSION. The results presented indicate that the pepsin concentration and output in response to liver extract applied to the gastric mucosa are of the same low order of magnitude as those evoked by histamine stimulation and do not approach the values which occur when a strong stimulus for pepsin secretion,

TABLE 1

Pepsin values of gastric juice from transplanted pouch after various stimulants

DOG NO.	LIVER PERFUSION			HISTAMINE		
	Volume	U/cc.	Pepsin output	Volume	U/cc.	Pepsin output
1	16.8	15	249	16.1	10	156
	18.3	10	175	14.4	9	131
	12.8	13	162	15.4	11	170
	15.8	14	221	14.7	17	245
Ave.....	15.9	13	202	15.2	12	175
2	3.3	23	77	5.5	8	44
	3.6	16	57	3.9	11	43
	3.0	39	116	3.4	13	44
	3.8	19	72	3.9	46	178
Ave.....	3.4	24	80	4.2	18	77

PILOCARPINE					
Dog 1			Dog 2		
Volume	Pepsin concentration	Pepsin output	Volume	Pepsin concentration	Pepsin output
7.1	74	524	4.0	135	542

such as pilocarpine, is used. It may be inferred that this type of hormonal gastric secretory mechanism is not responsible for the substantial increase in pepsin output noted during a meal. Some evidence exists that histamine *may* be the gastric hormone (7). The present results permit the assumption that as regards pepsin, histamine *could* be the humoral agent responsible for the type of secretion obtained by a presumably hormonal mechanism.

It is of interest to note that the type of humoral mechanism which may be presumed to operate during normal gastric secretion in response to a meal, does not cause a significant stimulation of pepsin secretion. Harper and Raper (8) have recently described a hormonal mechanism for the stimulation of pancreatic

enzymes. A humoral mechanism for pepsin secretion has never been clearly demonstrated.

SUMMARY AND CONCLUSIONS

It is concluded *a*, that stimulation of gastric secretion by a "hormonal" mechanism, such as liver extract in the stomach, does not cause a pronounced stimulation of pepsin secretion, and *b*, as regards pepsin, histamine *could* be the humoral or hormonal agent responsible for this type of secretion.

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THE ENZYME CONTENT OF PANCREATIC SECRETION FOLLOWING VARIOUS STIMULANTS

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Received for publication March 30, 1944

It has long been known that the composition of pancreatic secretion in response to secretin stimulation differs from that elicited by vagal excitation, the former producing a fluid of low enzyme content, the latter an enzyme-rich juice. Agencies which cause the pancreas to secrete, other than vagus excitation induced by electrical stimulation or by drugs, and secretin injection, include vasodilator substances and certain sympathomimetic drugs (1). The composition of pancreatic juice obtained in response to these last two stimuli has not been studied; therefore the experiments described below were performed in order to ascertain the possible mechanism whereby the sympathomimetic amines which stimulate secretion exert their effect.

EXPERIMENTAL. Intact dogs were prepared in the usual manner for collection of pancreatic juice under sodium pentobarbital anesthesia. The following materials were injected intravenously to elicit secretion. *a.* A standard secretin concentrate, SI, containing both secretin and pancreozymin. The amount used was 1 mgm. *b.* Prostigmine, which stimulates the pancreas as a result of vagus activity (2) in 0.5 mgm. doses. *c.* Sodium nitrite, in a dosage of 4 to 60 mgm. *d.* Epinine, one of the sympathomimetic amines which stimulates secretion, was given in a dose of 1 mgm.

Two injections of each drug were administered; the first served to flush out the animal's ducts and the cannula of the residual secretion from a previous stimulation, and this material was discarded; the juice obtained from the second injection was collected, measured, and analyzed for enzymes by the methods described elsewhere (3). When animals evidenced a spontaneous secretion before any stimulant was administered, this was also collected and analyzed.

RESULTS. The enzyme analyses on the samples obtained in response to the various stimuli revealed that prostigmine and sodium nitrite injections produced a secretion of high enzyme content, resembling closely in composition the spontaneous secretion; the prostigmine secretion in most cases evidenced the greatest enzyme concentration. Both SI and epinine evoked the production of a relatively enzyme-poor secretion.

The detailed data are listed in table 1. It may be noted from this table that the individual variation in response to any given stimulus is wide, but that the responses to all stimuli deviate in the same direction in each animal; and that any alteration in enzyme concentration is manifested equally by all three of the chief enzymes. The range of values encountered and the average responses are tabulated in table 2.

TABLE 1

Volume output and enzyme content of pancreatic secretion to various stimulants

DOG NO.	ORDER OF COLLECTION	DRUG INJECTED	VOLUME SECRETION	AMYLASE $K = \frac{1}{t} \log \left(\frac{a}{a-x}\right)$		LIPASE (CC. 0.5 N NaOH)		TRYPSIN (MGM. TYROSINE)	
				Per cc.	Total	Per cc.	Total	Per cc.	Total
			cc.						
1	1	SI	3.0	8.5	25.5	7.3	21.9	1.4	4.2
	2	Epinine	2.0	4.5	9.0	3.7	7.4	0.7	1.4
	3	Prostigmine	0.3	9.8	2.9	9.3	2.8	1.7	0.5
2	1	SI	1.5	5.5	8.25	4.4	6.6	1.1	1.65
	2	Epinine	2.1	3.3	6.9	1.7	3.6	0.9	1.9
	3	Prostigmine	2.9	10.7	31.0	6.5	18.9	4.0	11.6
3	1	Spontaneous	0.6	10.3	6.2	7.4	4.4	0.9	0.5
	2	SI	1.2	9.7	11.6	5.2	6.2	0.6	0.7
	3	Epinine	1.4	9.9	13.9	5.0	7.0	0.6	0.8
	4	Prostigmine	0.4	11.0	4.4	7.1	2.8	0.9	0.6
	5	$NaNO_2$	0.35	11.3	3.9	7.4	2.6	0.95	0.25
	6	SI	1.8	9.3	16.7	4.6	9.7	0.3	0.5
4	1	$NaNO_2$	0.75	7.8	5.8	8.5	6.4	0.87	0.66
	2	SI	0.5	3.6	1.8	6.5	3.25	0.18	0.09
	3	Epinine	0.5	5.0	2.5	7.2	3.6	0.27	0.14
	4	Prostigmine	0.3	8.2	2.2	8.8	2.4	0.90	0.24
5	1	Epinine	0.5	7.0	3.5	5.0	2.5	0.84	0.42
	2	SI	0.8	6.2	4.9	5.3	4.2	0.63	0.54
	3	Prostigmine	0.5	8.8	4.4	9.7	4.9	1.23	0.62
	4	$NaNO_2$	0.4	7.6	3.0	9.5	3.8	1.14	0.45
6	1	Spontaneous	0.25	12.6	3.15	4.9	1.2	1.31	0.34
	2	Prostigmine	2.0	15.0	30.0	5.8	11.6	1.42	2.84
	3	SI	0.5	3.0	1.5	3.0	1.5	0.93	0.46
	4	Epinine	0.25	3.6	0.9	3.3	0.8	0.96	0.24
	5	$NaNO_2$	0.5	7.5	3.8	4.8	2.4	1.11	0.56
7	1	SI	0.75	12.1	9.1	13.3	10.0	0.80	0.60
	2	Prostigmine	1.5	13.7	20.6	16.3	24.5	1.10	1.65
	3	Epinine	0.5	11.1	5.5	10.9	5.5	0.70	0.35
	4	$NaNO_2$	0.75	12.0	9.0	11.0	8.2	0.70	0.53
8	1	Spontaneous		6.6		12.4		1.09	
	2	Prostigmine	0.75	9.9	7.4	12.8	9.6	1.20	0.90
	3	SI	0.5	7.8	3.9	12.8	6.4	1.23	0.62
	4	Epinine	0.5	3.0	1.5	9.8	4.9	0.51	0.26
9	1	Spontaneous		8.8		14.8		1.85	
	2	Prostigmine	0.75	8.7	6.5	13.8	10.4	1.62	1.22
	3	SI	0.5	8.1	4.1	9.8	4.9	0.92	0.46
	4	Epinine	0.25	8.1	2.0	9.2	2.3	0.50	0.13
10	1	Epinine	0.5	8.3	4.2	5.6	2.8	0.20	0.10
	2	SI	1.0	11.3	11.3	7.4	7 4	0.48	0.48
	3	$NaNO_2$	0.5	14.0	7.0	9.4	4 7	1.25	0.63
	4	Prostigmine	1.0	16.2	16.2	12.0	12.0	2.69	2.69

DISCUSSION. It is evident from the data submitted that the pancreatic response to epinine is, on the basis of composition, indistinguishable from that elicited by SI, a secretin concentrate which contains both secretin and pancreozymin, presumably in the proportion in which they co-exist in the duodenal mucosa. However, it should be emphasized that this cannot be construed to indicate that a similar mechanism is involved in the two stimuli. Secretin stimulation is essentially unaffected by atropine and is abolished by ergotamine; the reverse is true for the sympathomimetic amines (1). It is also obvious that epinine cannot stimulate the pancreas by way of the vagus, for although its effect is prevented by atropinization, it yields a secretion of entirely different composition than that elicited by vagus excitation. Thus excluding the very remote possibility that the vagus contains separate fibers stimulating enzyme and fluid production by the pancreas which are unequally affected by epinine, this nerve cannot be involved in this type of stimulation.

The very marked similarity in the secretion obtained in response to prostigmine and to sodium nitrite suggests the interesting possibility that the means

TABLE 2

Range and average responses in enzyme composition of pancreatic secretion in 10 dogs

STIMULUS	NO. OF DOGS	AMYLASE			LIPASE			TRYPSIN		
		Low	High	Ave.	Low	High	Ave.	Low	High	Ave.
Spontaneous	4	6.6	12.6	9.6	4.9	14.8	9.9	0.90	1.85	1.29
Prostigmine	10	8.2	16.2	11.2	5.8	16.3	10.2	0.90	4.00	2.68
Sodium nitrite	6	7.5	14.0	10.3	4.8	11.0	8.4	0.70	1.25	1.00
SI	10	3.0	12.1	7.6	3.0	13.3	7.5	0.18	1.40	0.83
Epinine	10	3.0	11.1	6.4	1.7	10.9	6.2	0.20	0.96	0.62

by which vagus excitation brings about a secretion of the pancreas is partly through vasodilatation. An increase in size of the pancreas during vagus stimulation has been reported (4) which may be due either to vasodilatation or to distention of the gland by juice which is secreted and dammed back by spasm of the ducts.

Whether the sympathomimetic amines exert their effect on the pancreas by way of secretory elements in the splanchnic nerves is a possibility which remains to be examined, but appears to be remote. Since stimulation is effected by some drugs totally devoid of vasoconstrictor action; since the effect is not decreased by ergotamine and is abolished by atropine; and since most sympathomimetic drugs inhibit the pancreas, it seems most likely that the sympathetic nervous system is not implicated in this type of pancreatic secretion, and that the drugs which stimulate probably do so by a direct effect on the acinar cells, in a manner similar to that of the secretin-pancreozymin combination existing in the SI preparation. Favoring such a view is the marked similarity in composition of the secretion elicited by the two agents.

SUMMARY AND CONCLUSIONS

The pancreatic secretions obtained in response to stimulation by prostigmine, sodium nitrate, epinine, and SI (secretin plus pancreozymin) have been studied for their enzyme composition. Sodium nitrate and prostigmine were found to produce an enzyme-rich secretion, epinine and secretin an enzyme-poor one; all enzymes determined were equally effected by the various stimuli. The evidence is believed to indicate that sympathomimetic amines which stimulate pancreatic secretion do not do so by way of the autonomic nervous system. The similarity in the secretion obtained in response to cholinergic and vasodilator stimuli is presumptive evidence that the vagus exerts its effect on the pancreas by producing vasodilatation.

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COMPARATIVE STUDIES OF THE RATES OF OXIDATION AND GLYCOLYSIS IN THE CEREBRAL CORTEX AND BRAIN STEM OF THE RAT

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Received for publication March 13, 1944

Since Meyerhof (1) showed that the anaerobic accumulation of lactic acid by muscle was inhibited in the presence of oxygen, the relationship between oxidation and glycolysis has been studied for many tissues. Warburg and others (2) found that excised cerebral tissue was capable of forming lactic acid anaerobically and to a slight extent aerobically. Later work disclosed aerobic glycolysis *in vivo* for the brain liberates small but significant amounts of lactic acid (3) and pyruvic acid (4) into the blood stream.

Craig and Beecher (5) studied the aerobic and anaerobic metabolism in the cerebral cortex, medulla and cord in adult cats and found that the rates of oxidation and glycolysis both decreased as the neuraxis was descended from cerebral cortex to medulla and from medulla to cord although the decrease in glycolysis was more profound. Other observations have disclosed that the oxygen intake of excised cerebral tissues is slow in the newborn, with a rapid rise to a maximum comparatively early in life, succeeded thereafter by a small decline (6, 7). The rate of glycolysis was also found to be slower in the newborn than in the adult (8). In the present paper an attempt is made to estimate the calories obtainable aerobically and anaerobically by the brain.

METHOD. The brains of rats of various ages from birth to over 500 days were divided into two parts: 1, the gray cerebral cortex and 2, all of the brain stem excluding the striatum. Using these parts in litter-mates, we determined the oxygen intake of 100 mgm. of tissue in the Warburg respirometer for 45 minutes in a medium of phosphate-buffered saline at pH 7.3 with glucose as a substrate. The lactic acid production of 200 mgm. of tissue was measured in terms of the carbon dioxide displaced in 45 minutes from a Ringer-bicarbonate medium in an atmosphere of 5 per cent carbon dioxide and 95 per cent nitrogen. All of these experiments were carried out with absolutely uniform techniques and with special care as to the timing of the various steps of the procedures to insure comparable results. Oxidation readings were commenced in each instance 25 minutes after decapitation and glycolysis readings 35 minutes after decapitation. Each point determined represented an average of three to six observations. The manometric measurement of glycolysis was compared with the chemical determination of lactic acid production by the colorimetric method of Barker and Summerson (9) and it was found that they varied together, the Barker and Summerson method giving slightly lower values.

In order to compare the energy formed aerobically with that elaborated anaerobically, the oxygen intake and the carbon dioxide displacement respectively as measured in the manometer were calculated in terms of the calories per hour per 100 mgm. of tissue. The utilization of 100 cmm. oxygen may produce 0.5 calorie; 100 cmm. of carbon dioxide are displaced by the formation of 0.4 mgm. of lactic acid, the equivalent of 0.128 calorie (10). These values were used for the calculations of the aerobic and anaerobic productions of energy respectively. Because the metabolism of tissues decreases steadily after excision, the rates of energy formation are undoubtedly faster immediately after sacrifice than at the time the readings were made. Also the rate of anaerobic energy production is somewhat higher in its relation to the aerobic than indicated in the present observations since, starting with the time of decapitation, glycolysis readings were begun 10 minutes later than oxidation measurement. Though the exact quantitative relationship may, therefore, be somewhat erroneous, nevertheless the relative changes are on the whole correct.

Results. The results for the two parts of the brain, 1, the cerebral gray, and 2, the brain stem exclusive of the corpus striatum, for the caloric production of oxidation and by glycolysis for rats of different ages are presented in the graph. Curves were drawn by inspection. Starting from a low level at birth, the aerobic energy rises to a maximum at about 6 to 8 weeks in the cortex, then falls rapidly until about the 13th week after which the decrease is not significant. In the brain stem the maximum is reached some time between the 4th and 7th weeks. The rate then decreases sharply until about the 15th week and then more gradually to old age. Glycolysis of the cerebral cortex accelerates greatly until about the 5th week and then more slowly for about 3 months. After this time it appears to decline slightly. The rate of glycolysis of the brain stem rises to a maximum at about 3 weeks and then decreases, precipitously at first, and then not so noticeably to old age.

Discussion. The values for the oxidations and glycolysis of the cerebral hemispheres and brain stem are presented in graphic form (fig. 1). The average of each set of observations is represented on the graph in terms of the age of the rat and the number of calories calculated to be produced by 100 mgm. of the rat's excised brain tissue, cortex or stem as the case may be. Curves have been drawn through the points of inspection. Curves *A* and *B* trace the aerobic energy production of the cortex and brain stem from birth to over 500 days; curves *C* and *D*, their anaerobic energy production for the same period. Curves *A* and *B*, therefore, represent the calories produced aerobically, or the caloric requirement of the excised tissue; the curves *C* and *D*, the energy elaborated by these excised tissues during anoxia. The differences between curves *C* and *A* for the cortex and *D* and *B* for the stem show the caloric deficit which would arise in these parts during anoxia, i.e., the difference between the caloric requirements of the tissues and the number of calories which can be provided anaerobically. The magnitude of the deficit indicates the rate at which the various cerebral areas are deprived of their full quota of energy. It is obvious that as the caloric deficit piles up, life becomes impossible during anaerobiosis. The

difference between the anaerobic and aerobic production, i.e., the deficit, is very low at birth. It rises rapidly thereafter, and although it is already large at three weeks, the peak is at approximately the 7th or 8th week, when it appears to be somewhat higher than at any succeeding age. An estimate of the actual rate at which the deficit mounts in early life is made by selecting three arbitrary points in the curve of the figure at 5 days, 50 days and 1 year, as listed in the table. The deficit in energy production during glycolysis has been calculated

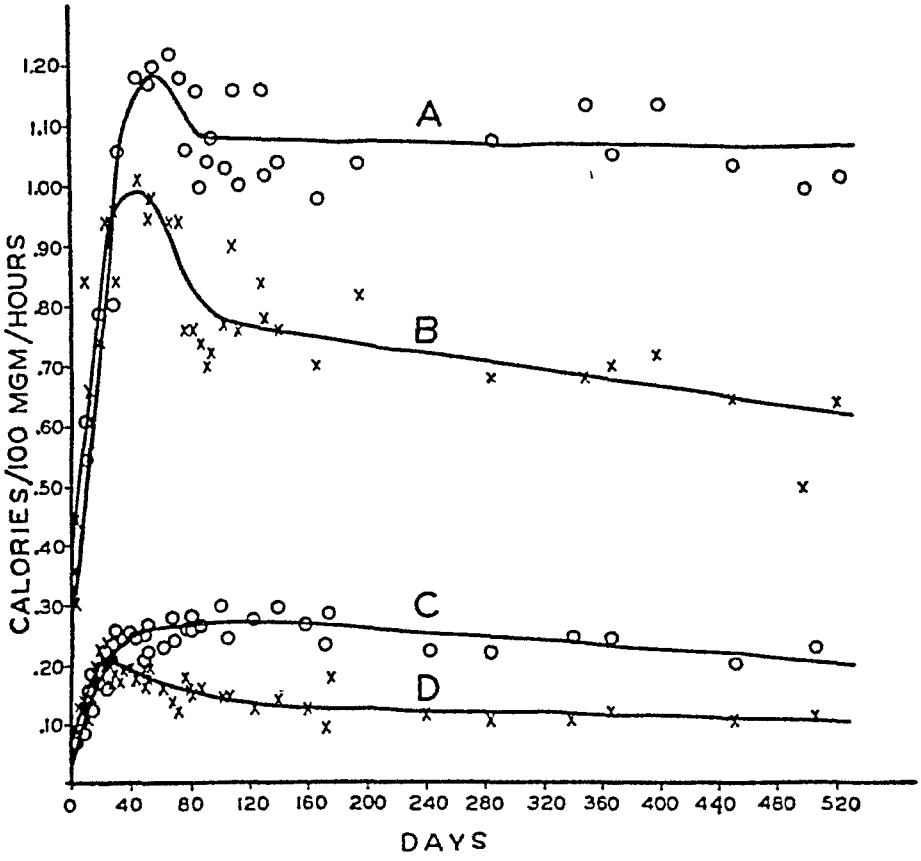

Fig. 1. *A* and *B* represent the aerobic energy production of the cerebral cortex and brain stem respectively; *C* and *D*, the anaerobic energy production of these same parts so that the difference between *A* and *C* and *B* and *D* indicate the anoxic caloric deficit in the upper and lower portions of the brain.

from these figures by substracting the values in curve *C* from those in *A*, and in *D* from those in *B*.

There is a decided increase in the deficit of both the cortex and the brain stem from 5 to 50 days and a noticeable decrease in the deficit in the stem after 50 days, although the decrease in the deficit in the cortex after 50 days is smaller and may not be significant. Probably for the brain as a whole the deficit would be somewhat less in old age than at 50 days. It is important to note that the cerebral cortex possesses a lower energy metabolism than the brain stem at 5 days and that the relative metabolic positions of these two parts are reversed by the 50th day.

At present we know of observations which may be explained by these results. Newborn rats may survive for an hour in an atmosphere of undiluted nitrogen (11), but the duration of survival becomes rapidly shortened and within 17 days almost reaches the short adult anaerobic survival period of approximately 2 minutes. The energy deficit of the newborn is much smaller than at any other time of postnatal life. Since the brain is the organ most sensitive to anoxia, the greater sensitivity of the adult can be seen chiefly to reside in the more rapid building up of this debt. The increasing energy debt during the first days of life accounts for the progressive curtailment of the anaerobic survival period. More specifically the maintenance of the vital activities depends on the medullary centers and the deficit in the adult brain, containing the medulla, is much greater than in the infant, accounting in part for the fact that the latter can continue to make respiratory movements so much longer in undiluted nitrogen. In the adult a larger deficit resides in the cerebral hemispheres than in the brain stem and perhaps this phenomenon is chiefly responsible for the greater susceptibility of

TABLE 1

Calories per 100 mgm. tissue per hour

AGE	OXIDATION		GLYCOLYSIS		DEFICIT	
	Cerebral cortex A	Brain stem B	Cerebral cortex C	Brain stem D	Cerebral cortex A–C	Brain stem B–D
days						
5	0.37	0.48	0.08	0.11	0.29	0.37
50	1.18	0.99	0.26	0.18	0.92	0.81
365	1.08	0.68	0.23	0.11	0.85	0.57

the rostral than the caudad portions of the adult neuraxis to anoxia or hypoglycemia (12, 13, 14, 15, 16, 17, 18). In contrast the data on the brain of the five-day-old infants suggest that on deprivation of energy the first injury occurs in the brain stem and that only after respiratory failure do the cephalad regions succumb.

SUMMARY

The rates of energy production, aerobic and anaerobic, of the cerebral cortex and the brain stem of rats ranging in age from newborn to 516 days were determined. The rates were found to increase rapidly in the first few weeks of life and then to show some decrease to old age. The difference between the aerobic and anaerobic energy production for each, the energy deficit sustained by the brain during anaerobiosis, is regarded as a factor determining the anaerobic survival period. This energy deficit indicates the rate at which the various parts of the brain are deprived of their full quotas of energy. The magnitude of the caloric deficit is correlated with 1, the tolerance of the infant to anoxia, and 2, the greater sensitivity of the rostral than other portions of the adult brain.

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BLOOD FLOW, PERIPHERAL RESISTANCE AND VASCULAR TONUS, WITH OBSERVATIONS ON THE RELATIONSHIP BETWEEN BLOOD FLOW AND CUTANEOUS TEMPERATURE[1, 2]

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Received for publication January 3, 1944

In studies of peripheral circulatory reactions, it is desirable to measure the peripheral resistance from time to time. It is also important to know what part changes in aortic pressure, in effective viscosity and in vasomotor activity have played in causing the observed alterations in peripheral resistance and blood flow. In this paper, the term vasomotor activity is used to designate the summated effects of vasomotor nerve activity and of *circulating* constrictor and dilator substances.

In experiments on the peripheral circulation in shock we measured the blood flow actually occurring in the tissues from time to time when they were receiving blood in the normal manner from the aorta. From these measurements we intended to appraise the alterations in vasomotor activity responsible for the observed changes. It shortly became apparent, however, that in experiments in which the mean aortic pressure was constantly changing, one could not assume that a proportional relationship existed between perfusion and blood flow in the absence of change of vasomotor activity. It therefore became necessary to determine under conditions of constant vasomotor activity what the influence of aortic pressure alone was. These requirements necessitated a study of the pressure-flow relationships under conditions corresponding to those used in the shock study. Two essentially different groups of studies were carried out, one in which arterial inflow was measured, and a second group in which venous outflow was measured. Both groups of studies will be reported in this paper.

The influences of perfusion pressure and of blood concentration and viscosity on flow in the isolated extremity of the dog have been analyzed in two previous papers,—one by Whittaker and Winton in 1938 (1), and one by Pappenheimer and Maes in 1942 (2, 3). Both groups of investigators measured the outflow while perfusing the extremities by means of an artificial system with blood and with saline solutions. The latter also studied the influence of epinephrine and of sciatic nerve block. Our results differ considerably from the former, but agree in part with the latter study. Our interpretations, however, differ considerably from both.

[1] Supported by a grant from the Commonwealth Fund.

[2] Preliminary reports of this work have been presented in Fed. Proc. 1: 32, 1942; Proc. Soc. Exper. Biol. and Med. 53: 228, 1943.

METHODS. The relationship between perfusion pressure and blood flow was studied by measuring the blood flow through various vascular beds in situ while perfusing them at a series of pressures from approximately zero to mean aortic pressure or above. This was done, *a*, while the region was in a control state; *b*, during periods of heightened vasomotor activity occurring spontaneously and induced by hemorrhage, and *c*, during periods of lessened vasomotor activity induced by section of the vasomotor nerves to the region. These perfusions were carried out in 63 vascular beds in 45 dogs anesthetized with morphine alone, or with morphine plus sodium barbital, sodium pentobarbital or chloralosane. The doses used were the same as those given in a previous paper (4). In each experiment, after completion of dissections and before beginning perfusion, the animal was given heparin[3] intravenously to prevent clotting. Two different groups of studies were carried out on these dogs:

1. Arterial inflow studies. In one group arterial inflow was recorded while the vascular bed was perfused from a reservoir by way of its cognate artery (5). Three types of vascular beds were so studied. *1.* The first of these was the vascular bed of the skin of the medial surface of the lower part of the thigh and the leg and the dorso-medial aspect of the foot. The saphenous artery, which is the cognate artery for this vascular bed, was perfused by way of a cannula inserted into the central stump of the left femoral artery distal to the saphenous branch, while the femoral artery was occluded centrally by a clamp. *2.* The second of these was the vascular bed of that portion of the quadriceps supplied by the branches from the proximal two-thirds of the femoral artery. This segment of the left femoral artery, which will be called the cognate artery for this vascular bed, was perfused by way of a cannula inserted into the stump of the left inferior epigastric artery, while the femoral artery was occluded by two clamps placed at the level of the inguinal ligament and just proximal to the saphenous branch. This vascular bed, and the preceding cutaneous vascular bed, were frequently perfused simultaneously. *3.* The third vascular bed studied was that of the gastrocnemius and soleus muscles. The cognate artery for this vascular bed was a branch of the left popliteal artery which gave off several side branches to the gastrocnemius and soleus muscles. This vessel was perfused by a cannula inserted in one of its terminal branches while the artery was occluded by a clamp at its point of origin from the popliteal artery.

In order to be sure that the measured inflow at all times indicated the correct flow through the vascular bed, all adjacent collateral vascular beds were simultaneously perfused at the same pressure. This was accomplished in the same manner for all of the above vascular beds. In each, the aorta was occluded by a clamp applied just below the renal arteries and the distal portion perfused at the same pressure as the cognate artery by way of a cannula inserted in the central stump of the right femoral artery.

On each vascular bed, one or more sets of simultaneous measurements of blood flow and perfusion pressure were made at a series of pressures from approximately zero to mean aortic pressure or higher. Perfusion was maintained at each pressure for 5 to 20 seconds. Between each 20 second perfusion period the vessels were connected with the aorta by releasing the above mentioned clamps. One to two minutes elapsed between each 20 second perfusion. Approximately 20 minutes were required for the complete set of determinations. Perfusion pressure was varied by altering the pressure on the surface of the blood in the reservoir. The reservoir held only enough blood for two or three 20 second perfusions. Frequent filling plus gentle agitation prevented settling of the blood. In order to check on the amount of settling that might have occurred, hematocrit readings were made on the reservoir blood at the end of each set of determinations. They never differed from the animal's blood by more than +2 to −4 cell volume per cent, and usually they were within

[3] The heparin used was Liquaemin which was kindly supplied by Roche Organon, Inc., Nutley, N. J. Approximately 0.3 ml./kgm. was given initially followed by 0.1 ml./kgm./half-hour thereafter.

0 to −1 cell volume per cent. In many experiments the entire apparatus was surrounded by a constant temperature air bath. This was not done in all experiments, however, since the small shifts of temperature of the blood in the reservoir were found to have very little influence on the rate of flow. Blood flow was recorded continuously throughout the perfusion period by an orifice and optical differential manometer (6, 7), and perfusion pressure by suitable optical pressure manometers (8) with a photokymograph. The orifice meter was carefully calibrated immediately preceding and following each set of pressure flow determinations using the same blood and calibrating at several rates of flow from the minimum to the maximum recorded in the experiment.

2. Venous outflow studies. In a second group of experiments, venous outflow was recorded from the vascular bed of the distal part of the whole hind extremity. This region was mechanically isolated by two wire ligatures applied approximately at the junction of the middle and lower thirds of the thigh so as to compress tightly all structures except the femoral artery and vein and the saphenous and sciatic nerves. Blood flow through the vascular bed was recorded by collecting for 30 seconds to one minute the outflow from the femoral vein. This was accomplished by way of a cannula inserted into the central stump of the saphenous vein while the femoral vein was occluded proximally to the branch, the tip of the outflow tube being placed at the zero pressure level of the femoral vein. In two of these experiments the femoral artery was perfused from a reservoir as above. In the remainder, the vascular bed was at all times supplied directly from the aorta and the various heads of pressure, recorded by an Hg manometer, were obtained by suitable compression of the femoral artery at the level of the wire ligatures by a special clamp. In the latter experiments, perfusion was maintained at each pressure for 1 to 2 minutes before measuring flow. The clamp was released after each measurement. Thirty to forty minutes were required for a complete set of determinations.

Additional details on the above methods are given in a previous paper (5). In each experiment the viscosity of the blood was measured at frequent intervals and at several rates of flow with a viscosimeter of 0.3 mm. bore (9, 8). Perfusion pressure will be expressed in terms of the arterio-venous difference of pressure. In most of the experiments the pressure in the femoral vein was 4 to 6 mm. Hg.

Technical complications encountered in these experiments included: change in hematocrit reading and viscosity from time to time, exchange of blood between the cognate and collateral vascular beds (5), and the occurrence of spontaneous rhythmic changes in vascular tonus. In most of the experiments the rate of flow remained constant throughout the perfusion period. In a few, however, flow either increased or decreased during the first few seconds of perfusion at each pressure before becoming stable. Only those experiments in which the viscosity remained within ±4 per cent of the control reading and the hematocrit remained within +2 to −6 cell volume per cent of the control reading, and in which collateral exchange of blood was controlled, will be analyzed.

RESULTS. *A. Mean blood flow.* Blood flows at initial mean arterial pressures in a number of experiments are reproduced in table 1. These figures are somewhat smaller than the rate of 10 to 15 ml./min./100 grams which H. C. Wiggers (10) found for the body as a whole, but they are quite close to the value of 3.9 ml./min./100 grams which Barcroft and Edholm (11) observed in the fore-arm in man. Comparison of the second and third rows suggests that under the conditions of these experiments the blood flow in the skin of the distal half of the hind leg is a sizable proportion of the total flow in this part of the extremity.

Subcutaneous temperature was recorded in the perfused area with a Leeds and Northrup micromax using iron-constantin needle electrodes (4). The difference between the subcutaneous and air temperatures expressed as per cent of the difference between rectal and air temperatures correlated quite well with

the flow per 100 grams of extremity. This is illustrated in table 1 and in the data given in the legends to the various figures.

B. Relationship between perfusion pressure and blood flow in skin. Satisfactory data on the relationship between perfusion pressure and blood flow through the vascular bed of the skin of the medial surface of the lower thigh, the leg and the foot, supplied by the saphenous branch of the femoral artery, were obtained in 14 dogs. The results were essentially the same in all experiments. Those from typical experiments are reproduced in figures 1 and 2.

C. Relationship between perfusion pressure and blood flow in muscle. The relationship between perfusion pressure and blood flow through the vascular bed of the quadriceps muscle supplied by muscular branches of the femoral artery between the inguinal ligament and the saphenous branch was studied in nine satisfactory experiments. Studies were made on the vascular beds of the gastrocnemius and soleus muscles in seven experiments. The results in the

TABLE 1

REGION	NO. DOGS	INITIAL BLOOD FLOWS, MEAN ART. PRESS., AND CUT. TEMPS.								
		Blood flow in ml./min./100 gm.*			Mean arterial press.			$\left(\frac{CT}{ReT - AT} \times 100\right)$†		
		Max.	Min.	Av.	Max.	Min.	Av.	Max.	Min.	Av.
Quadriceps muscle....	10	13	3.0	6.9	159	68	123			
Distal portion of hind leg (wire ligature)...	10	5.6	1.7	3.2	165	72	107	80	15	60
Skin supplied by saphenous branch of femoral artery.....	11	4.3	0.4	1.6	159	100	108	60	25	40

* In the case of the skin supplied by the saphenous artery the flows are expressed in terms of the flow per 100 grams of extremity distal to the origin of the saphenous branch.

† CT = subcutaneous temperature; ReT = rectal temperature; AT = air temperature.

latter were, however, less satisfactory because of the difficulty in preventing exchange of blood by way of the prominent anastomotic communications with the collateral vascular beds. The relationship between pressure and flow in most of the experiments on muscle was similar to that for skin. However, in two experiments on the quadriceps muscle the relationship of pressure to flow had the characteristics shown in figure 3.

D. Relationship between perfusion pressure and blood flow in the distal portion of the hind extremity isolated by wire ligatures. Satisfactory studies were made in 13 extremities in 9 dogs of the relationship between perfusion pressure and blood flow through the vascular beds of the lower third of the thigh, the leg and the foot. In the two experiments in which the femoral artery was perfused from a reservoir the results were essentially the same. Those from one of the experiments are reproduced in figure 4. In the remaining 11 areas the different heads of pressure were obtained by partial compression of the femoral artery. In these, flow was not measured until after perfusion had been maintained for 1 to 2 minutes. The results were, in all cases, similar to those reproduced in figure 5.

Discussion. *I. Critique of the work of other investigators.* In recent years two other studies of the relationship of perfusion pressure to flow in the hind limb of the dog have been made. The first of these was done by Whittaker and

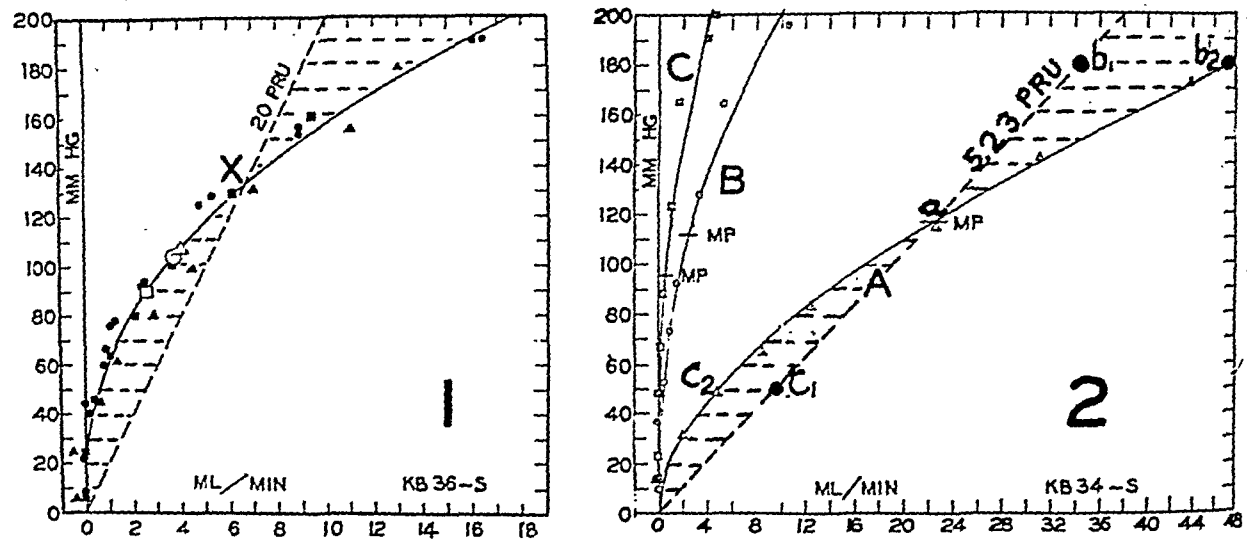


Fig. 1. Plot of relationship between perfusion pressure and blood flow through the skin supplied by the saphenous artery, in the hind leg of a dog in which vasomotor activity remained practically constant at a high level. Dog weight, 19 kgm. Anesthesia, morphine 2 mgm./kgm. and chloralosane, 50 mgm./kgm. Arterial inflow recorded, perfusion from a reservoir. Solid triangles ▲—data obtained between 4:30 and 4:47 p.m.; solid circles ●—data obtained between 10:35 and 10:50 p.m.; solid squares ■—data obtained between 12:50 and 1:05 a.m. Hollow symbols indicate level of animal's mean arterial pressure. Temperatures of perfused area of skin in the three periods were respectively 30.1, 29.5 and 30.2°C. These were higher than the air temperature by 27, 28 and 23 per cent of the difference between air and rectal temperatures. Hematocrit readings of the dog's blood were respectively 51.8, 51.0, 46.2 per cent. Solid line is plot of equation $F = \left(\frac{P}{60.4}\right)^{2.395}$. Dashed line = loci of pressure − flow determinations which would yield a peripheral resistance of 20 *PRU* $\left(1\ PRU = \frac{1\ \text{mm./Hg}}{1\ \text{ml/min.}}\right)$. For significance of shaded area see text p. 532. Ordinate scale = perfusion pressure in millimeters Hg, expressed as the difference in pressure between arterial and venous pressures. Abscissal scale = blood flow in ml./min.

Fig. 2. Plots of the relationship between perfusion pressure and blood flow through the skin supplied by the saphenous artery in the hind leg of dog in which a spontaneous progressive increase in vasomotor activity occurred. Dog weight, 20 kgm. Anesthesia—morphine 2 mgm./kgm and sodium barbital 125 mgm./kgm. Arterial inflow measured, perfusion from a reservoir. A—triangles △—control—3:23 to 3:38 p.m., *B*—circles ○—9:17 to 9:40 p.m., *C*—squares □—11:55 p.m. to 12:20 a.m. Temperatures of perfused area of skin were respectively 34.6, 32.6 and 31.7°C. These were higher than the air temperature by 45, 30 and 20 per cent of the difference between air and rectal temperature. Hematocrit readings were respectively 54.8, 53.7 and 52.8. *MP*—level of mean arterial pressure. For other lettering see figure 1 and text page 532.

Winton (1) on the isolated limb perfused with defibrinated blood. Besides being denervated, this preparation also differed from ours in that it was necessary for them to allow 2 and preferably 3 hours to elapse during which the limb was

perfused with the defibrinated blood in order to allow the limb to accommodate itself to the conditions of artificial perfusion before beginning measurements. Their study was primarily concerned with determining the effect of the per cent of red cell composition on the apparent viscosity of the blood; and, with the exception of noting that the blood flows ranged from 80 to 400 ml./min. at 100 mm. Hg perfusion pressure in the different preparations, they made no attempt to study the effects of change of vascular tonus.

Whittaker and Winton's results differ from ours chiefly in the fact that their pressure-flow data plot on straight lines intercepting the pressure axis at around 10 to 20 mm. Hg when using a normal percentage red cell composition, and in

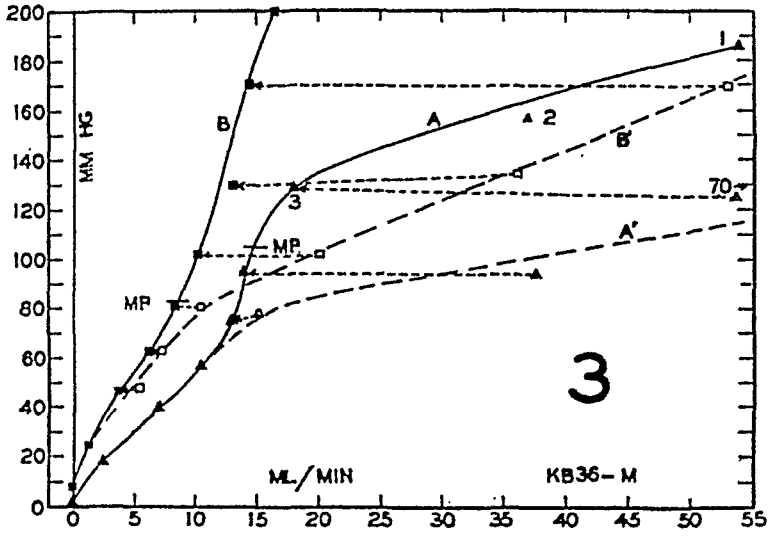
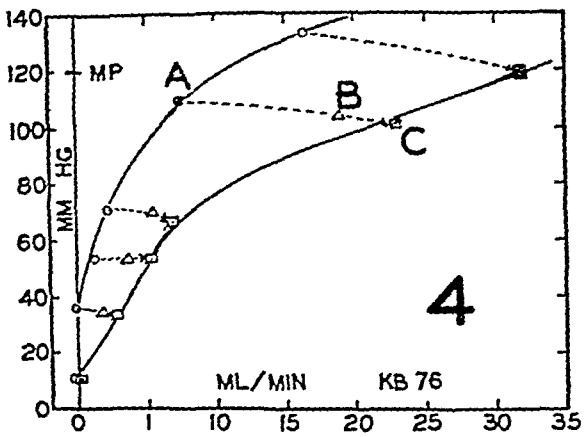


Fig. 3. Plots of relationship between perfusion pressure and blood flow in the portion of the quadriceps muscle supplied by the branches of the femoral artery between the inferior epigastric and saphenous branches. Same animal as figure 1. Plots *A* and *A'* recorded simultaneously with plots indicated by triangles and plots *B* and *B'* recorded simultaneously with data indicated by squares in figure 1. *A'* and *B'*—dashed lines—flow during first 2 seconds of perfusion at each pressure; *A* and *B*—solid lines stabilized flows after maintenance of perfusion pressure for 10 to 20 seconds. Arrows—change of flow with time at each perfusion pressure. Note: For technical reasons, a 5 to 6 second interval of complete ischemia immediately preceded each 20 second perfusion period. For other lettering see figure 1.

the very high rates of flow which they obtained in their preparations. They do not give data on the mass of tissue perfused. According to our own measurements, the hind legs of their 8 to 10 kgm. dogs would weigh approximately 700 grams. This would give flows of 11 to 58 ml./100 gm./min. or far in excess of those which we obtained and far greater than those for the body as a whole. We believe that these excessive rates of flow were due to vascular dilatation induced in part by the 0.1 per cent chloral hydrate in their perfusion fluid. In addition they made no attempt to ligate the cut ends of the collateral arteries supplying the hind leg, believing that these had minimal connections with the femoral artery which was perfused. Studies reported previously (5) show, however, that the flow between cognate and collateral arteries across precapillary anasto-

motic channels may amount to far more than the flow through the cognate capillary bed when the pressure in the distal stump end of the severed collateral arteries is dropped to zero, as was the case in their experiments. It seems possible that the vasodilatation plus the occurrence of flow across the precapillary



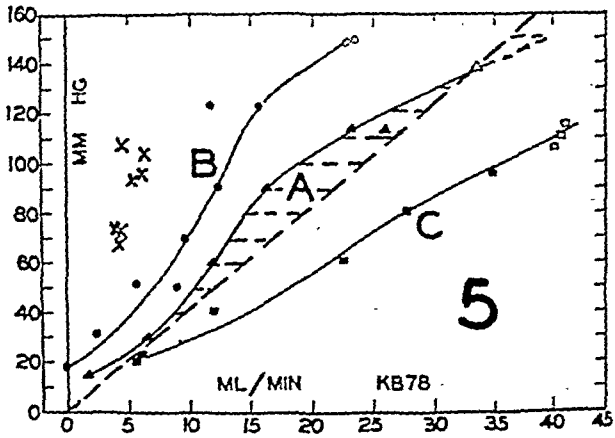


Fig. 4. Plots of perfusion pressure and blood flow through the portion of the hind leg supplied by the femoral artery distal to the saphenous branch. Dog weight 15 kgm., anesthesia, morphine 5 mgm./kgm. This region was mechanically isolated from the remainder of the body by two tight wire ligatures placed at about the level of the saphenous branch. Perfusion from a reservoir. Points labeled *A* (circles) were the rates of arterial inflow during the first 5 seconds of perfusion at each pressure; points labeled *B* (triangles) were the rates of inflow at about 45 seconds to 1 minute after starting perfusion and points labeled *C* (squares) were the inflows at the end of 1½ to 2 minutes of perfusion. The arrows indicate the progressive change of flow with time at each perfusion pressure. Duration of registration—2:27 to 3:00 p.m. Hematocrit 59 per cent. Other lettering as in figure 1.

Fig. 5. Plots of the relationship between perfusion pressure and blood flow in a vascular bed similar to that used for figure 4. Dog weight 14.5 kgm., anesthesia—morphine 8.2 mgm./kgm. Perfusion from the aorta, perfusion pressure controlled by variable compression of the femoral artery. *A*—triangles—control (1:00 to 1:40 p.m.); *B*—circles—after an increase in vasomotor activity induced as a part of temperature regulatory reactions (see reference (4)) (4:00 to 4:50 p.m.); *X*'s—after a hemorrhage equal to 23 ml./kgm. (9:30 to 10:00 p.m.); *C*—squares—after reduction of vasomotor activity by sectioning the sciatic nerve at mid-thigh (11:30 p.m. to 12:10 a.m.). Prior to the nerve section the blood had been reinfused and the plot of pressure vs. flow had returned to the position indicated by plot *A* (11:00 to 11:20 p.m.). Hollow symbol = determinations at mean arterial pressure, solid symbols—determinations at reduced pressures obtained by partial compression of the femoral artery. Hematocrit readings for plots *A*, *B*, *X* and *C* were respectively: 62.5, 61.5, 55.4 and 60.0. Cutaneous temperatures in paw of perfused leg were respectively: 35°, 34.5, 30 and 36.3°C; these were greater than the air temperature by 81, 68, 37 and 86 per cent of the difference between rectal and air temperatures. The rectal temperature rose from 37.5 to 38.8 between the first and second set of determinations. For other lettering see figure 1.

anastomotic channels may have been responsible in part for the occurrence of plots which approach a straight line. In addition, they used only very limited ranges of pressure (40 through 88; 60 through 120) which provide, we believe, insufficient data upon which to make the claim that there is a linear relationship between perfusion pressure and blood flow.

The second perfusion study was carried out by Pappenheimer and Maes (3) on innervated hind limbs of dogs perfused with defibrinated blood. Their preparation differed from ours in that at no time did the leg receive blood from the animal, but only blood recirculated through a lung-pump circuit. They also studied only the whole hind limb, whereas we perfused isolated muscle and skin in addition to the whole hind limb. In addition, we extended our range of perfusion pressures over a wider range than they used, particularly at the lower ranges. In their studies they compared the rates of flow of a saline solution with those of blood and observed the effects of nerve block by cooling the sciatic nerve and the effects of various concentrations of epinephrine.

Pappenheimer and Maes found that the plots of perfusion pressure vs. flow for saline solutions were linear and since they extrapolated back to the origin, it may be concluded that a proportional relationship existed between perfusion pressure and flow. When studying blood flow, however, they found that with increasing perfusion pressure the blood flow was augmented proportionally faster than the pressure until rates of flow of 20 to 40 ml./min. (20 to 140 mm. Hg pressure) were reached. As a result, the plots were convex towards the pressure axis. Above these rates of flow the plots became approximately linear. When the "straight line" portions of the plots were extrapolated back to zero flow, however, they intercepted the axis at pressures of 10 to 80 mm. Hg; thus, while the plots were apparently linear, they did not indicate a proportional relationship between perfusion pressure and blood flow even at high perfusion pressures and rates of flow.

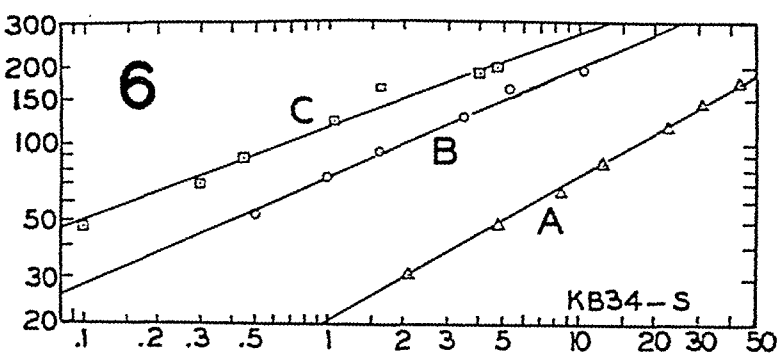
It is not possible to gauge exactly what their rates of flow per mass of tissue were, but according to their observation that the total weight of the perfused portion of the extremity in a "typical preparation" was 351 grams, the rates of flow at perfusion pressures approximating normal mean arterial pressures with the nerve not blocked would be: for a perfusion pressure of 100—from 5.7 to 57 ml./min./100 grams tissue; and for a perfusion pressure of 150 mm. Hg—from 11.3 to 95 ml./min./100 grams of tissue. The last figure was obtained by extrapolation of their data and is admittedly inaccurate, but was necessitated by the fact that in this experiment the maximum perfusion pressure used was only 70 mm. Hg.

The extremely high rates of flow in comparison with our figures and those of Barcroft and Edholm (11) suggest that again in Pappenheimer and Maes' study the vascular beds were abnormally dilated or else that ours were abnormally constricted. We believe that the former is the true state of affairs and that a possible cause of this might be the absence from their perfusion fluid of some chemical substance present in normally circulating blood which serves a purpose of maintaining the tonus of the blood vessels and that in the absence of this substance the vessels become abnormally dilated. It might be argued that, in those of our experiments in which the blood was withdrawn and placed in a reservoir prior to perfusion, a constrictor substance was formed. However, such argument cannot be used for those experiments in which flow was measured by collection of the venous outflow while the vascular bed was perfused directly

from the aorta and in these experiments the rates of flow ranged only from 1.7 to 5.6 ml./min./100 grams of extremity.

If we accept that the vascular beds studied by Pappenheimer and Maes were abnormally dilated then we may harmonize their experimental results with ours since at maximal dilatation we approached "a straight line relationship" between flow and perfusion pressure and since under conditions which we would regard as normal, that is in their "constricted" extremities, their data may be plotted on parabolic curves.

II. Physical factors influencing blood flow; pressure-flow relationship during states of constant vascular tonus. The regular increase in the increments of flow per increment of perfusion pressure in figure 1, in plots A, B and C in figure 2,



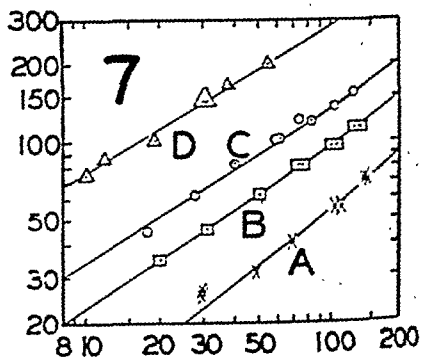


Fig. 6. Log-log plots of the data reproduced in figure 2. Ordinate scale—perfusion pressure. Abscissal scale—blood flow. In A: $F = \left(\frac{P}{20.3}\right)^{1.765}$; in B: $F = \left(\frac{P}{74}\right)^{2.315}$; and in C: $F = \left(\frac{P}{116}\right)^{2.735}$.

Fig. 7. Log-log plot of data from an experiment on blood flow in the hind leg of a dog, reported by Pappenheimer and Maes. This Journal **137**: 187, 1942. A, B, C and D—plots at progressively increasing vascular tonus induced by epinephrine. Ordinate scale—perfusion pressure in millimeters Hg, abscissal scale—blood flow in millimeters per minute. In A: $F = \left(\frac{P}{2.08}\right)^{1.41}$; in B: $F = \left(\frac{P}{5.53}\right)^{1.59}$; in C: $F = \left(\frac{P}{8.62}\right)^{1.68}$, and in D: $F = \left(\frac{P}{20.9}\right)^{1.78}$.

in plots A′ and B′ in figure 3, and plot A in figure 4 suggests that the relationship between flow and pressure may be represented by a relatively simple family of equations. This is confirmed by plots of the data on log-log paper. Such plots were made on 6 of the experiments in which a regular relationship between pressure and flow were observed and in each case the data could be approximated by the equation $F = \left(\frac{P}{C}\right)^n$, where F = flow in milliliters per minute, P = arterio-venous difference of pressure, C = a constant and n = an exponent greater than 1. The data for figure 2 are replotted on log-log paper in figure 6. In figure 7 we have replotted on log-log paper the data from one of Pappenheimer and Maes' (3) experiments on the hind limb of the dog. Over the range of flows which they studied, their data also plot as a series of straight lines with a slope less than 1/1.

Three possible explanations may be offered for the parabolic character of the plots relating perfusion pressure and flow:

(1) Poiseuille's law (12) for laminar flow of homogeneous liquids through tubes states that

$$Q = P \cdot R^4 \cdot K,$$

where Q is the volumetric rate of flow in milliliters per minute, P is the difference in pressure between the two ends of the tube in grams per centimeter, R is the radius of the tube in centimeters and $K = \frac{\pi}{8\mu L}$, where μ is the absolute viscosity of the liquid in gram seconds per square centimeter, and L is the length of the tube in centimeters. If the radii of the blood vessels which provide the principal frictional resistance to the flow of the blood should increase with rising internal pressure then the volumetric rate of flow would be a function of P^n, where n is greater than 1 and might approach a value of 5.

The observations of Whitaker and Winton (1) and of Pappenheimer and Maes (3) that the flow of saline through the hind leg is very nearly proportional to P^1 appear to deny the possibility that the blood vessels which offer the principal resistance to flow are dilated with increase of perfusion pressure. However, one can argue that in their saline experiments the normal mechanical distention of the vessels with rising perfusion pressure was prevented at high perfusion pressures by the elevation in extravascular pressure which would result from the increase in tissue fluid and tension in the absence of the osmotic pressure normally provided in the plasma proteins. It should also be noted that Fleish (13) in an earlier study observed a parabolic relationship between perfusion pressure and saline flow in the fresh, surviving rabbit kidney. He also found that while a linear relationship might be found between saline flow and perfusion pressure in the hind legs of the frog, the plot of flow vs. pressure was changed to a parabola by the addition of a small amount of adrenalin.

(2) Since blood is a non-homogeneous liquid, the effective viscosity may be expected to become progressively less with increasing heads of pressure (and velocity of flow) (14, 15, 1), on account of the red cells becoming grouped more or less as a solid rod in the center of the stream as the velocity of flow is raised. According to the observations of Whittaker and Winton (1), the maximum effect of this shift of distribution of the cells in the flowing stream for bloods of 50 per cent red cell volume (the average for our experiment) would be a reduction of the viscosity by a factor of 1.47 (2.2/1.5). In figure 1, the flow increased from 0.5 ml./min. at a pressure of 45 to 13.6 ml./min. at a pressure of 180. If the only factor operative in addition to the increase of perfusion pressure had been the decrease of effective viscosity and assuming that the maximum possible change in viscosity occurred between these two pressures, the flow could have increased only from 0.5 to 2.94 ml./min. It is apparent that the relationship between perfusion pressure and flow in these experiments cannot be explained on the basis of such changes of effective viscosity alone.

(3) It may be assumed that at low perfusion pressures red cells pass through some arterioles and capillaries but not through others and that with increasing perfusion pressure progressively more capillaries are irrigated. Knowledge of whether this actually occurs or not will have to await studies of perfusion pres-

sure and blood flow combined with microscopic observations of capillary behavior. This concept is, however, the equivalent of (1) above. It is probable that all three of the above factors contributed to the observed relationship between perfusion pressure and blood flow in our studies as well as in those of Whittaker and Winton and those of Pappenheimer and Maes.

In the papers of Whittaker and Winton and of Pappenheimer and Maes, much stress is placed on the occurrence of a linear relationship between perfusion pressure and blood flow especially above certain minimal rates of flow. A linear relationship should signify that throughout the range of pressures in which it occurs: no further vascular dilatation, no further change in effective viscosity, nor any opening of new capillaries is occurring. However, under these conditions, flow would become not only a linear function of pressure, but it would also become proportional to pressure. In other words, the straight line would project back to the origin. Such a situation would be equivalent in figure 1 to having flow increase progressively faster than pressure, i.e., along the solid line from zero pressure to point X, until the vascular dilatation and change of effective viscosity have become maximal. The plot would then swing up and follow the dashed line marked 20 *PRU*. Since in none of our data (with the apparent exceptions in figures 3 and 5 below mean arterial pressure) nor in that of Pappenheimer and Maes, or of Whittaker and Winton, do the plots ever become so directed that the apparent straight line portion can be extrapolated back to zero, it is apparent that either the effective viscosity decreases or the lumen of the vessels increases continuously up to the maximum perfusion pressures used. On this basis, the straight lines drawn by Whittaker and Winton and by Pappenheimer and Maes may be only apparently straight lines drawn tangent to a limited segment of a parabola distal from the vertex.

In view of the above discussion, we believe that in regions where a parabolic relationship holds between perfusion pressure and blood flow purely physical factors have operated, namely, a progressive decrease in effective viscosity and/or a progressive mechanical dilatation of the blood vessels with rising perfusion pressure. According to this concept, vasomotor activity, vascular tonus and extravascular pressure have remained constant in these vascular beds regardless of the perfusion pressure used.

III. Physiological factors influencing blood flow. The discussion in section II provides possible explanations for the regular increase in the increments of flow per increment of pressure in small tubes. In fact, it is not easy to see how any other relationship could occur on the basis of purely physical principles. The sigmoid character of the relationship between pressure and flow seen in plots A and B in figure 3, in plot C in figure 4 and in plots A and B in figure 5 is apparently due to the operation of some additional factor or factors.

In the case of the venous outflow experiments, represented by figure 4, it might be assumed from the above discussion that the flow would have increased proportionally to some power greater than 1 of the perfusion pressure had a steady perfusion pressure been used. In these experiments, however, the perfusion pressure, supplied by the aorta and varied by the degree of compression of the

femoral artery by a clamp, became progressively more pulsatile in character as mean aortic pressure was approached. This might be expected to cause larger flows at the higher perfusion pressures than those anticipated from constant pressure perfusion (14). This possibility was studied in 12 dogs not included in the above results. In these dogs, flow into the popliteal artery was recorded *a*, while the artery was supplied by the pulsatile head of pressure provided by the aorta, and *b*, while it was perfused under a steady head of pressure equal to mean aortic pressure. The popliteal artery was perfused by way of a cannula inserted into the central stump of the saphenous artery while the femoral artery was occluded by a clamp central to the branch. Blood flow was recorded with the orifice and differential manometer described under methods. During both types of perfusion the collateral vascular beds were perfused from the aorta. In 26 comparisons in these experiments the pulsatile flows were sometimes greater than and at other times less than the steady pressure flows and the differences between them were no greater than could be accounted for by the probable error in measuring pulsatile flows.

A more likely explanation of the sigmoid shape of the plots reproduced is that at perfusion pressures below mean aortic pressure reactive vasodilatation occurred which tended to maintain the normal irrigation of these vascular beds despite the lowered perfusion pressure. This explanation is supported by the following two observations:

1. In the experiment reproduced in figure 3 it is observed that in the vascular bed of the quadriceps muscle the rate of flow at the higher perfusion pressures rapidly diminished after the first 2–3 seconds to a lower rate which was maintained throughout the remainder of the 20 second perfusion interval (plot A′ to plot A). At the lower perfusion pressures the rate of flow remained at the initial rate throughout the 20 second perfusion period. In the same experiment, blood flow in the skin supplied by the saphenous artery, measured simultaneously with the above, remained constant throughout each 20 second perfusion period, and the plots of perfusion pressure vs. flow were typical parabolic curves (fig. 1). We interpret plots A and A′ of figure 3 as follows. The basic tonus due to vasomotor nerve activity probably would have yielded a parabolic plot of pressure vs. flow which would have passed through points 1 to 3 of plot A. As a result of the 5 second period of ischemia preceding each perfusion, however, the local vascular tonus was temporarily reduced to a level such that the plot was shifted to the position A′. At the lower perfusion pressures this diminished vascular tonus tended to persist. At the higher perfusion pressures and rates of flow the products of metabolic activity producing vasodilatation were rapidly removed and the local vascular tonus restored during the latter part of each 20 second perfusion period to the basic level provided by the tonic vasomotor nerve activity.

2. In the experiment illustrated in figure 4, the vascular bed of the distal part of the hind leg was isolated by wire ligatures and inflow was recorded at various intervals after the start of perfusion. At all perfusion pressures the rate of flow increased during the first minute of perfusion. At the lower perfusion pressures, however, the percentage increase of flow was much greater than at the higher perfusion pressures. The plot of perfusion pressure vs. flow during the first 5 seconds of perfusion (line A) is a typical parabola. The plot of perfusion pressure vs. flow at the end of 1½ to 2 minutes of perfusion (line C) is becoming sigmoid in shape, like the various plots in figure 4 and like plot A in figure 3.

The relatively greater flows at low perfusion pressures in these experiments may also be due in part to a lowering of the extra vascular pressure by osmotic

absorption of the tissue fluids when low perfusion was maintained for a sufficient length of time. It is perhaps significant that sigmoid shaped plots were obtained only in preparations in which all or at least part of the vascular bed supplied muscle, and in which the rate of flow was measured only after perfusion had been maintained for a relatively long time at each pressure.

IV. Effects on pressure-flow relationships of change of vasomotor activity. In each of the figures 2, 3 and 5, plot B is displaced to the left of the initial or control plot A. This shift was due, we believe, to an increase in vasomotor activity. In some experiments the heightened vasomotor activity occurred spontaneously, in others it was induced by hemorrhage. In figure 3, plot C is displaced to the right. This was due to a reduction in vasomotor activity following section of the sciatic nerve at about the middle of the thigh.

In the log-log plots of all the experiments in which the pressure-flow relationships were similar to those in figures 1 and 2, it was noted as shown in figure 6 that not only were the B plots displaced to the left, but also the slope of the straight line joining the points was less steep. Conversely, in most instances in which a decrease in vasomotor activity was produced by section of the appropriate nerve, the log-log plot not only was displaced to the right but become steeper, approaching a slope of 1:1. It is evident from the log-log plots (fig. 7) of the data from one of Pappenheimer and Maes' experiments, that they observed similar changes. The decreased slope of the log-log plots with increased vascular tonus may be due to a change in the characteristics of the blood vessels which allows them to become relatively more ideally distensible, or it may be caused by a greater change in effective viscosity with velocity of flow as the vessels become narrowed by the increased vasomotor activity.

As illustrated in figures 3 and 5, in all experiments and conditions of perfusion in which a sigmoid relationship between pressure and flow was noted, this sigmoid shape, and particularly the convexity to the right at low perfusion pressures, was preserved and even augmented as a result of increased vasomotor activity. On the other hand, as shown in figure 5 the sigmoid character became less prominent as a result of section of the sciatic nerve. If the sigmoid shape is due to the occurrence of a reactive vasodilatation at low perfusion pressures then this diminution of vascular tonus due to the production of local metabolic products would appear to be relatively more effective the higher the general level of vasomotor activity.

V. Peripheral resistance. Measurements of peripheral resistance have been used considerably in recent years in studying the peripheral circulation. In all cases where the term has been used in a quantitative sense it represents the ratio of the arterial pressure (or the arterio-venous difference of pressure) to the rate of flow through the organ being perfused. In studies of the whole animal the term total peripheral resistance (TPR) has frequently been employed, but the significance is the same; that is, TPR = the ratio of the mean aortic pressure to the total blood flow (cardiac output) (15 – 21). Most of these authors convert the simple ratio $\frac{\text{mm.Hg}}{\text{ml/min}}$ to $\frac{\text{dyne}\cdot\text{sec}}{\text{cm}^5}$ by multiplying the former by $\frac{1330\cdot 60}{\text{cm}^2}$ and

consider that $\frac{1 \text{ dyne} \cdot \text{sec}}{\text{cm}^5} = 1$ absolute unit, A.U. We can see no advantage to this latter mode of expression since it is not as readily appreciated as the direct expression and since it does not in any way increase the accuracy. We therefore propose that the unit of peripheral resistance (or of TPR) be $\frac{1 \text{ mm.Hg}}{1 \text{ ml/min}}$ (see also Pappenheimer and Maes, 3) and suggest the designation *PRU* for this unit.

Peripheral resistance so defined is proportional to $\frac{\mu \cdot L}{R^4}$ in Poiseuille's equation (see p. 527). It is a measure of the totality of all factors which affect the blood flow, i.e., the effective viscosity of the blood; the lengths of the vessels; and, their individual and collective cross-sectional areas, as determined by the intrinsic tone of the blood vessels, the constant play of vasomotor nerve impulses, the presence of constrictor and dilator substances and the extravascular pressure provided by the tissue tension. In view of the multiplicity of factors which may affect peripheral resistance, it remains to show to what extent changes of peripheral resistance may be used to interpret vasomotor activity.

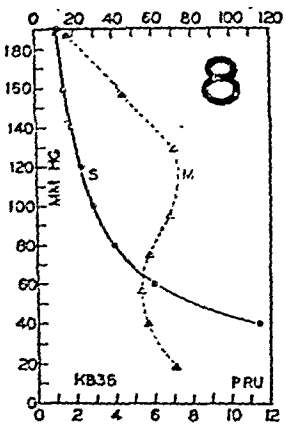
VI. Interpretation of direction of change of vasomotor activity from measurements of peripheral resistance. As long as the arterial pressure remains constant the fluctuations of peripheral resistance correctly indicate the direction and qualitatively the magnitude of the alteration of vasomotor activity (see p. 533). The following examples indicate, however, that peripheral resistance varies whenever the aortic (or perfusion pressure) is altered even when vasomotor activity remains constant.

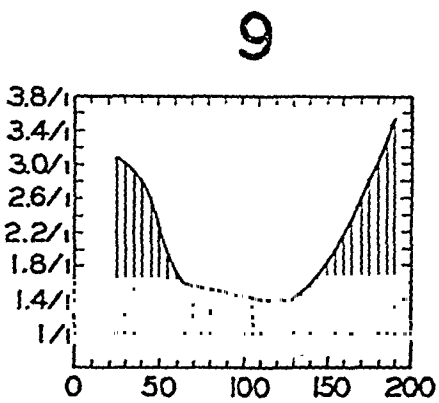
a. In the determinations for skin flow in the experiment reproduced in figure 1, we believe that the vasomotor nerve activity, circulating humoral constrictor substances and the vascular tonus remained constant throughout the determinations. However, the peripheral resistance decreased regularly from 115 *PRU* at a perfusion pressure of 40 mm.Hg to 28 *PRU* at a pressure of 100 mm.Hg and to 11 *PRU* at a perfusion pressure of 190 mm.Hg. This is illustrated graphically by the solid line *S* in figure 8.

b. In the control determinations for muscle flow in the same experiment, plotted as line *A* in figure 3, we believe that the vasomotor activity remained constant, but that vascular tonus was relatively low at low perfusion pressures, increased regularly as the perfusion pressure approached mean arterial pressure and remained constant thereafter despite further elevation of perfusion pressure. The plot of peripheral resistance vs. perfusion pressure for this same experiment, reproduced as line *M* in figure 8, indicates that from 20 to approximately 60 mm.Hg perfusion pressure the peripheral resistance decreased from 7 to 5.3 *PRU*, that from 60 to 110 mm.Hg the resistance increased again to 7 *PRU* and from 110 to 170 mm.Hg it decreased steadily again to 1.6 *PRU*.

Of more importance, however, is the question of interpretation of changes of peripheral resistance in the presence of an alteration of vasomotor activity associated with rise or fall of aortic pressure. During the control period in the

experiment illustrated in figure 2, the aortic pressure was 116 mm.Hg, the blood flow at this pressure was 22.2 ml./min., and the peripheral resistance at this pressure was 5.23 *PRU*. These pressure-flow data are represented by point *a*, and the peripheral resistance by the line 5.23 *PRU*. Now let us suppose that during an ensuing experimental period the aortic pressure rose to 180 mm.Hg. If at this time a flow of 34.4 ml./min. were obtained (point b_1) computation would indicate that the peripheral resistance had *not changed*, yet obviously the vasomotor activity would have been increased significantly. If a flow of 47.5 ml./min. were obtained (point b_2) it would indicate that the peripheral resistance had declined to 3.79 *PRU*, i.e., a 28 per cent change, yet in this





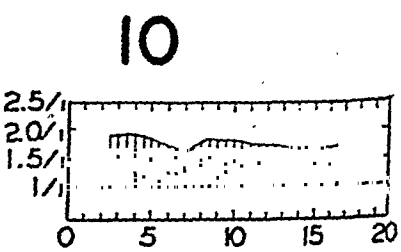


Fig. 8. Plots of variation of peripheral resistance with perfusion pressure. Circles (*S*)—plot for solid line in figure 1 (skin). Triangles—plot for line A in figure 3 (muscle). Ordinate scale—perfusion pressure. Abscissal scale—peripheral resistance in *PRU* (1 *PRU* = 1 mm. Hg./ml. per min.), scale at top applied to circles, scale at bottom to triangles.

Fig. 9. Graph of the ratio of peripheral resistance in the constricted state, curve *B* in figure 2—to the peripheral resistance in the control state, curve *A* in figure 2 at the same perfusion pressure—plotted against the perfusion pressure. Ordinate scale—ratio—PRU_B/PRU_A; abscissal scale—perfusion pressure in millimeters Hg.

Fig. 10. Graph of the ratio of peripheral resistance in the constricted state—curve *B* in figure 2—to the peripheral resistance in the control state—curve *A* in figure 2 at the same rate of flow—plotted against the rate of flow. Ordinate scale—ratio PRU_B/PRU_A—abscissal scale—flow in ml./min.

case *no change* in vasomotor activity would have occurred. If the flow were anywhere between 34.4 and 47.5 at this pressure, it would indicate a *decrease* in peripheral resistance, but an *increase* in vasomotor activity. With flows greater than 47.5 or less than 34.4 vasomotor activity and peripheral resistance would change in the same direction.

In a similar manner, if the aortic pressure were to fall to say 50 mm.Hg and a flow of 9.55 ml./min. (point c_1) were obtained, it would indicate *no change* in peripheral resistance but a *decrease* in vasomotor activity. A flow of 4.84 ml./min. (point c_2) would indicate an *increase* in peripheral resistance to 10.4 *PRU*, i.e., a 100 per cent change, but *no change* in vasomotor activity; and any flow between 4.8 and 9.55 ml./min. would indicate a *decrease* in vasomotor activity, but *an increase* in peripheral resistance. With flows less than 4.8 or

greater than 9.55, vasomotor activity and peripheral resistance would change in the same direction. The loci of the pressure-flow points at which the change in peripheral resistance is the opposite of the change of vasomotor activity are shown by the shaded areas. Similar loci are indicated by the shaded areas in figures 1 and 5.

On the basis of the above argument, it is evident that *whenever arterial (or perfusion) pressure is altered: (a) peripheral resistance may change in the absence of any modification of humoral or vasomotor nerve activity, and (b) in the presence of a combined alteration of aortic pressure and of vasomotor activity peripheral resistance may not indicate the degree of the change and may not even tell the direction of the change of vasomotor activity unless one knows what modification of peripheral resistance would have been produced by the observed alteration of aortic pressure alone.*

While it is not possible to reason too closely from results in isolated vascular beds to the behavior of the circulatory system as a whole, it is at least reasonable to assume from the above observations that caution must be used in attempting to interpret changes of vasomotor activity in the body as a whole from computations of total peripheral resistance in the presence of a concomitant rise or fall of aortic pressure, unless in some way it is possible to know what change in total peripheral resistance would have resulted from the observed alteration of aortic pressure alone.

VII. Quantitative expression for change of vasomotor activity. The most satisfactory method for studying quantitatively change of vasomotor activity would seem to be to determine during a control period and again during the experimental period the peripheral resistance at each of a series of perfusion pressures. This is essentially what Pappenheimer and Maes did, and is the basis upon which these experiments were started. However, the method is laborious, and it is difficult to be sure that, during a given experimental period, the vasomotor activity has remained constant for the 20 to 45 minutes necessary for making the required complete set of pressure-flow determinations. Furthermore, it is difficult to portray in a time graph the successive changes in the plots relating pressure and flow. Pappenheimer and Maes avoid the latter difficulty by computing a ratio of the slope of the "straight line" portion of the plot for the experimental period to the slope of the "straight line" portion of the control plot of pressure vs. flow. We have not been able to avail ourselves of this method since in our experimental data almost any slope can be obtained for either the control or experimental periods depending upon what portion of the data one chooses to draw the straight line through. In situations in which the pressure-flow data fit the equation $F = \left(\frac{P}{C}\right)^n$, change in vasomotor activity could be expressed in terms of the variation of the constant C and the exponent n.

Change in vasomotor activity might be expressed in terms of the ratio of the peripheral resistance in the experimental period to that at the same perfusion pressure in the control period (PRU_e/PRU_c ($Pe = Pc$)[4]). Such comparisons

[4] It should be noted that this ratio is equal to Fc/Fe where Fc is the rate of flow in the control period and Fe is the rate of flow at the same perfusion pressure in the experimental period.

were made by Pappenheimer and Maes (3) and have been made in most of our experiments. Both studies indicate, however, that the ratio varies greatly with the head of pressure used. For instance, in the experiment in figure 2 the ratio PRU_e/PRU_c ($Pe = Pc$) for the experimental state represented by line B and the control state represented by line A varied from 6.2/1 at a perfusion pressure of 170 to 11.5/1 at a perfusion pressure of 55 or an increase of 85 per cent. Similarly, in figure 3, PRU_e/PRU_c ($Pe = Pc$) for the control state represented by line A and the experimental state represented by line B is 3.5/1 at 190 mm.Hg, drops to 1.4/1 at 130 mm.Hg and rises again to 3.1 at 25 mm.Hg. The latter data are illustrated graphically in figure 9. On the basis of these facts it is apparent that the ratio of the peripheral resistance in the experimental period to that at the same perfusion pressure in the control period indicates qualitatively the direction of the change of vasomotor activity but fails to give a satisfactory quantitative measure of the magnitude of the change.

Change in vasomotor activity may also be expressed in terms of the ratio of the peripheral resistance in the experimental period to that at *the same rate of flow* in the control period (PRU_e/PRU_c ($Fe = Fc$)[5]). This comparison has also been made in most of our experiments and has been found to be much more constant over a wide range of flows than the ratio computed at equal perfusion pressures. For example, in figure 2 the ratio varied only from 2.66/1 at a flow of 15 ml./min. to 3.4/1 at a flow of 1 ml./min. or an increase of 27 per cent as compared with the 85 per cent increase for the ratio computed at equal pressures. In the experiment reproduced in figure 3, the ratio PRU_e/PRU_c ($Fe = Fc$), presented graphically in figure 10, is much more constant throughout the complete range of flows than is the corresponding ratio at equal pressures presented in figure 9. Apparently Pappenheimer and Maes did not compare the peripheral resistance in the constricted state with that in the unconstricted state at the same rates of flow. Our analysis of their published data shows, however, that in their experiments also the ratios at the same rates of flow are much more constant over the entire range of flows, than are the corresponding ratios made at the same pressures over the entire range of pressures.

Conclusions. In view of the discussion presented in this paper, it is our conclusion that no method for expressing change of vasomotor activity yet devised rests on a completely logical basis. The most satisfactory *practical* expression for change of vascular tonus due to vasomotor nerve activity, and for the appearance of constrictor or dilator substances in the blood stream, is the ratio of the peripheral resistance in the experimental period to the peripheral resistance measured in the control period *at the same rate of flow.*

SUMMARY

Vascular tonus is defined as the active contraction of the muscular walls of the small blood vessels and is considered to be influenced physiologically by vaso-

[5] This ratio is of course equal to Pe/Pc, where Pe = the aortic (or perfusion pressure) at which the flow was measured in the experimental period and Pc is the perfusion pressure required in the control period to cause the same rate of flow.

motor nerve impulses, humoral substances and metabolic products. The summated effects of the first two are defined as the vasomotor activity. Changes in vascular lumen in response to altered intraluminal pressure per se are considered to be a physical reaction and not to represent a change in vascular tonus.

The relationship between vasomotor activity, peripheral resistance, blood flow and subcutaneous temperature was studied by recording either the arterial inflow or the venous outflow at a series of perfusion pressures in various vascular beds in the hind limbs of anesthetized dogs. These studies were made during control states and during periods of increased vasomotor activity occurring spontaneously and induced by small hemorrhages, and during periods of decreased vasomotor activity induced by sectioning the sciatic nerve. Average blood flows at mean arterial pressure were 6.9 ml./100 grams of muscle. In the combined skin and muscle of the lower half of the hind extremity the average flow was 3.2 per 100 grams of extremity and in the skin of the latter the average flow was 1.6 ml./100 grams of extremity.

Only experiments in which the hematocrit reading and viscosity of the blood remained within +4 to −6 per cent of the control values and in which collateral circulation artifacts were avoided were analyzed. During a constant state of vasomotor activity the increments of blood flow per increment of perfusion pressure in skin and often in muscle increased regularly from zero upward when flow was measured immediately after establishment of the perfusion pressure. The relationship between perfusion pressure and flow in these experiments could be represented by the equation $F = \left(\frac{P}{C}\right)^n$, where n has values between 1 and 5, usually about 2. This relationship appears to be explainable on a purely physical basis. When the rate of flow was measured after 1 to 2 minutes after establishment of the perfusion pressure in muscle and in the whole distal part of the extremity the flows at pressures below mean arterial pressure appeared to be relatively greater than they would be on the basis of a purely physical relationship between pressure and flow. This was probably due to a vasodilatation induced locally by the accumulation of metabolic products as a result of the slowed flow at low perfusion pressures, but might also be due to a lowering of the extravascular pressure. The result was a sigmoid rather than a parabolic shaped curve relating perfusion pressure and flow.

In all experiments, increase of vasomotor activity decreased the rate of flow at each perfusion pressure. In those experiments in which the data fitted the equation $F = \left(\frac{P}{C}\right)^n$, increase of vasomotor activity increased the magnitude of the constant C and of the exponent n.

Peripheral resistance, physically, is the ratio of the perfusion pressure to the flow through the vascular bed. The term PRU is proposed as a unit of peripheral resistance, where $1\ PRU = \frac{1\ \text{mm.Hg}}{1\ \text{ml/min}}$. Peripheral resistance was found to be altered when the perfusion pressure was raised or lowered, even when vasomotor activity remained constant. Conversely, change of vasomotor activity

was not in all cases correctly indicated by an appropriate alteration of peripheral resistance.

Various methods of expressing change of vasomotor activity were discussed. It was concluded that the only completely satisfactory method is to compare the plot relating pressure to flow over the complete range of pressures from zero to mean aortic pressure in a control period, with a similar plot obtained in the experimental period. However, this method is laborious and it is difficult to depict the progressive changes in these plots with time. As a compromise the next most satisfactory method appears to be to determine during a control period the relationship between pressure and flow over the entire range of flows anticipated during the subsequent experimental period and thereafter to make only single determinations of pressure and flow, usually at a perfusion pressure equal to the existing mean aortic pressure. The results may then be expressed in terms of the ratio of the peripheral resistance (or perfusion pressure) in the experimental period to the peripheral resistance (or perfusion pressure) observed during the control period at the same rate of flow.

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THE EFFECT OF HISTAMINE AND HCl ON GASTRIC SECRETION AND POTENTIAL[1]

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Received for publication March 4, 1944

In a previous paper (11) it was shown that there was no maintained depolarization of the gastric potential in the resting stomach when the potential was shunted through comparatively low external resistances. It was suggested on the basis of these observations that the electromotive force of the stomach might be able to deliver inside the stomach a quantity of electrical energy equal to the amount of energy necessary for the production of the hydrochloric acid of the gastric secretion. This obviously, taken by itself, cannot be considered proof that electrical energy actually furnishes the energy for the production of the HCl. Of the possible further experimental approaches to this problem the next logical one, in the writer's opinion, is a study of the relationship between the potential difference across the stomach as measured by surface electrodes and the secretion of HCl. A review of the literature reveals that numerous workers have been interested in this problem. Their reports, however, are extremely conflicting. Sarre (14) reported that in cats anesthetized with pernoston (an anesthetic under which adequate gastric secretion is obtained) there was an increase in the negativity of a mucosal electrode with respect to an electrode on an injured muscle in the thigh when the stomach was stimulated to secrete. On the other hand, Mislowitzer and Silver (9) also used pernostonized cats and found a decrease in the magnitude of the potential difference across the stomach after administration of histamine. Quigley, Barcroft, Adair and Goodman (10), using pouch dogs, reported that there was no relationship between the gastric potential and secretion. Swyngedauw (15) claimed that in humans a change in potential was associated with the stimulation of gastric secretion. With the exception of Sarre, none of these investigators measured the secretory rate of the stomach. Sarre measured the pH of fluid in contact with the mucosa but did not attempt to determine the secretory rate at intervals in a given experiment. The primary purpose of the present paper is to investigate further this relationship. A method was devised in which the potential difference across the stomach and the rate of secretion of hydrogen ions could be measured from the same portion of the stomach and the composition of the fluid in contact with the mucosa conveniently controlled.

METHODS. Dogs fasted for 24 to 30 hours were anesthetized with pernoston[2] (80 mgm. per kgm., subcutaneously). The stomach was exposed through an

[1] This investigation was aided by a grant from the Joseph E. Seagram and Sons Company.

[2] The Riedel-de Haen Company, New York, kindly supplied the pernoston used in these experiments.

abdominal opening and an incision was made in the stomach midway between the lesser and greater curvatures. A portion of the stomach (along the greater curvature) was placed between a lucite chamber and a ring of lucite (see fig. 1). The lucite ring was about 1.5 mm. thick and had an oblique cut in it so that the blood vessels supplying the portion of the stomach in the chamber could be slipped inside it. The stomach was fastened tightly between the chamber and the ring by screws. The chamber thus formed had a volume of approximately 20 ml. The area of the mucosa inside the chamber was 21 cm.^{2} Fluids could be introduced into the chamber and replaced by means of tubes T_1 and T_2. E_m represents the electrode used for leading off the potential from the mucosa. Contact with the solution in the chamber was made by means of a saturated KCl solution. The tip of the tube containing the saturated KCl solution pro-

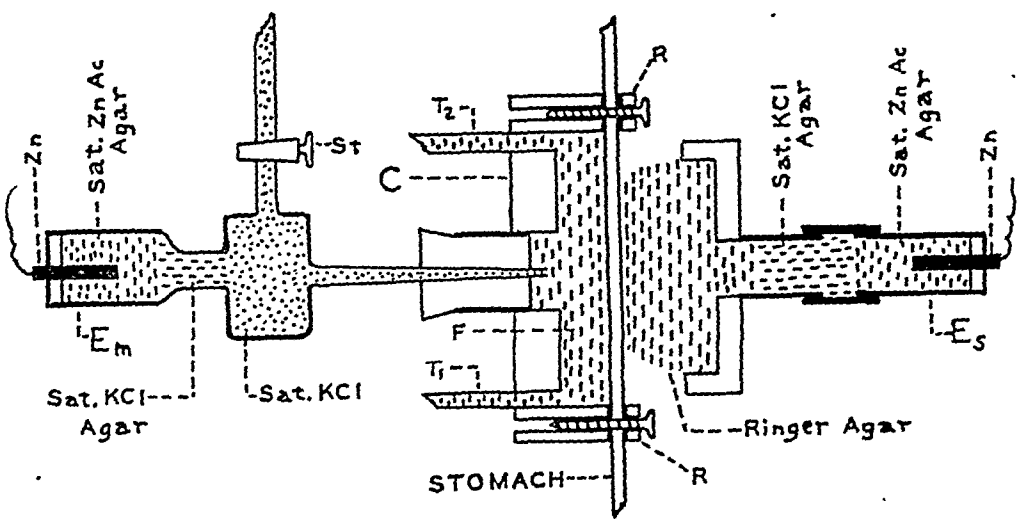

Fig. 1. Schematic drawing of a sagittal section of apparatus with stomach in place. R refers to the lucite ring which contains eleven holes for the screws used in clamping the ring to chamber, C. F refers to the fluid in the chamber. The blood vessels supplying the portion of the stomach in the chamber are not shown in the diagram.

truding into the chamber was approximately 0.5 mm. in diameter. This tube could be flushed out with fresh KCl by opening stopcock S_t. Control experiments revealed that this arrangement was as effective in eliminating liquid junction potentials (to within a fraction of a millivolt) as one in which a tube 5 mm. in diameter was substituted for the capillary tube. It is assumed, therefore, that the potential at the junction between a saturated KCl solution and any of the solutions used in these experiments is zero (1) (but see MacInnes and Longsworth (7)).

The diameter of the Ringer agar portion of the serosal electrode, E_s, in contact with the serosa was made less than the diameter of the stomach inside the ring R in order to avoid interference with the blood supply. The potential differences were measured with a potentiometer. The potential difference between the two electrodes with the stomach in the chamber, minus the potential difference between the two electrodes when they were placed in the same solution of 0.9

per cent saline, is referred to in this paper as the potential of the stomach. The error due to variation in the potential of the electrodes was less than 1 mv.

The rate of secretion of hydrogen ions was determined in the following manner. The chamber was filled with 0.9 per cent saline and at the end of 10 minutes it was drained and washed with enough 0.9 per cent NaCl to make a total volume of 100 ml. The pH and total acidity of the sample were determined. The pH was measured with a Beckman pH meter, the total acidity was determined by titration with a standard NaOH solution using phenolphthalein as the indicator. The secretory rate was calculated in terms of milligrams of HCl per 10 minutes (total acidity of sample minus total acidity of same amount of saline). Calculations of the secretory rate from pH measurements gave values that were essentially the same as those obtained from the total acidities. Only a negligible fraction of the titratable acidity was, therefore, present in a form other than that of free hydrogen ions.

All solutions introduced into the chamber were warmed to a temperature of 39 ±1°C. A small amount (about 0.1 ml.) of saturated KCl was washed through the capillary tip of the mucosal electrode whenever an HCl solution was placed in the chamber and occasionally when saline was replaced with more saline. It was found that flushing the capillary tip in this way was not followed by an appreciable change in the potential.

Evidence that the ability of the stomach to secrete HCl is not seriously impaired under the conditions of these experiments is found in the fact that with histamine stimulation over 9 mgm. of HCl may be secreted by that portion of the stomach in the chamber in a 10 minute period. Histamine was administered subcutaneously at regular intervals unless otherwise specified.

Results. *Secretory rate and potential before histamine.* After the stomach had been placed in the chamber it was washed with large quantities of 0.9 per cent NaCl. After this procedure the rate of secretion of hydrogen ions was practically zero. The pH of the 100 ml. samples was above 5.0 after a 10 minute interval. The possibility that hydrogen ions were being secreted but were neutralized by a buffered transudate or secretion was tested by comparing the acidity of the fluid in the chamber (titrated to a pH of 9.0) with that of the original saline. It was found that the titratable acidity of the fluid in the chamber was not greater than that of the original saline by more than 0.08 mgm. of HCl. The potential was found to have the same orientation as that found in previous experiments (11, 12), i.e., the serosa was positive in the external circuit to the mucosa. There was a tendency for the potential to increase with time after the beginning of an experiment. The potential reached a relatively constant value of from 70 to 95 mv. in about one hour after the beginning of an experiment. An average value of 71 mv. was given for the resting potential in a preliminary report of this work (13). This lower value was due to the fact that in some of the early experiments histamine was administered before the potential had reached a relatively constant level. The lower values of the potentials reported in another paper (11) were probably due to the use of a different anesthetic in some experiments and to poorer condition of the animals.

It was found that the resting potential was influenced by the concentration of NaCl (being higher with concentrations less than 0.9 per cent and lower with higher concentrations of saline). However, all the experiments reported in this paper were performed with 0.9 per cent NaCl in the chamber unless otherwise specified.

Effect of histamine on potential and secretion. Subcutaneous injection of histamine (0.05 to 0.1 mgm. per kgm. of the diphosphate every 10 minutes) was followed by a marked decrease in the potential to a new level. As shown in figure 2 (a typical experiment) this decrease in potential was associated with the onset of secretion. After the new level of potential had been reached further administration of histamine had very little effect on it. On the other hand,

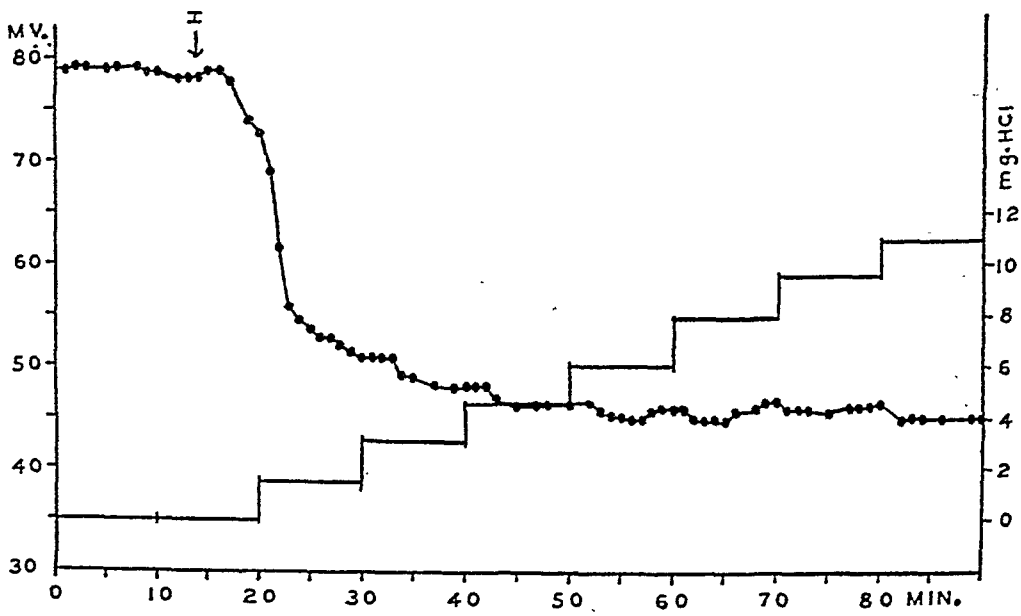

Fig. 2. Effect of histamine injections on the potential and secretory rate. Closed circles represent potential in mv., secretory rate is in milligrams of HCl per 10 minutes. At arrow marked *H*, 1 mgm. of histamine diphosphate was given subcutaneously and this dose was repeated at 10 minute intervals for the duration of the experiment.

the secretory rate continued to increase after the potential had reached the new level. In experiments in which a single dose of histamine was given the typical changes in potential and secretion were observed and after a period of time the secretory rate gradually decreased to zero and the potential increased in magnitude to approximately its original level. If the single dose was relatively small (0.005 to 0.05 mgm. per kgm. of the diphosphate) the potential did not fall to as low a level as it did following the larger doses. Further histamine stimulation was then followed by a further lowering of the potential.

Influence of the pH of the fluid in the chamber on the potential. Since HCl solutions of the concentrations present in the gastric secretion can give rise to diffusion potentials of considerable magnitude, the following experiments were designed to determine the effect of the pH of the fluid in the chamber on the potential.

a. *Effect of pH changes due to gastric secretion.* When the stomach was actively secreting the pH of the fluid in the chamber decreased from that of the original saline (approximately 6) to 1.9 to 2.6 in a 10 minute period. There was no evidence that a definite change in the potential was associated with this decrease in the pH of the fluid in the chamber. This is illustrated in figures 2 and 4 since fresh saline was placed in the chamber every 10 minutes. The greatest change in any of the experiments after replacing the fluid with fresh saline was 3 mv. (fig. 3B).

b. *Effect of a buffer on potential after histamine.* The possibility that the change in potential after histamine might be modified by a buffered solution in the chamber was tested with an isotonic phosphate buffer of a pH of approxi-

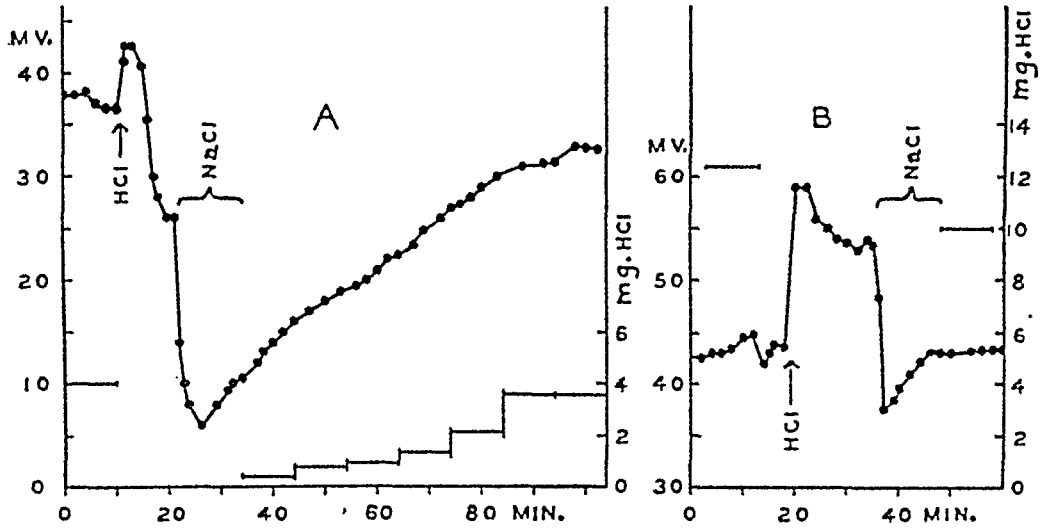

Fig. 3 (A and B). An HCl solution of a pH of 0.75 was placed in the chamber at the time indicated by the arrows. Saline (0.9 per cent) was washed through the chamber during the time indicated by the brackets. The flow of the saline solution was stopped during measurement of the potential. Histamine diphosphate, 1 mgm. every 10 minutes subcutaneously, was administered in both experiments.

mately 7.2. This solution was placed in the chamber and after histamine stimulation the potential decreased to about the same extent as with saline in the chamber. The magnitude of the potentials after histamine in the four experiments performed with the phosphate buffer after they had become relatively stable were 45, 38, 37 and 38 millivolts. (Compare with column 3 of table 1.) The phosphate buffer remained in the chamber until the potential reached a relatively constant value and the pH of the buffer at the end of the experiments was 6.1 or higher, except in one experiment in which the buffer remained in the chamber for a much longer period of time.

c. *Effect of HCl solutions.* The HCl secreted by the stomach diffuses into the fluid in the chamber and the potential produced by this diffusion (since it occurs in an aqueous solution outside the stomach) would be oriented toward the mucosal electrode, i.e., opposite to the potential of the stomach. In an attempt to determine to what extent this diffusion potential contributes to the decrease in

the potential after histamine stimulation, the effect of solutions of HCl on the potential was determined. These experiments were performed with solutions of HCl varying in pH from 0.64 to 1.03. After the stomach had been stimulated with repeated injections of histamine and the potential had reached its new level, approximately 200 cc. of the HCl solution was run into the chamber and the pH of the last 50 cc. of the solution leaving the chamber via tube T_2 was measured. The pH of this solution was equal to that of the original HCl to within 0.02 of a pH. Figures 3 and 4 show three of these experiments in which the saline in the chamber was replaced with an HCl solution. In these experiments the potential showed an increase before the HCl had stopped flowing through the chamber. The fact that the potential remained near the maximum for at least

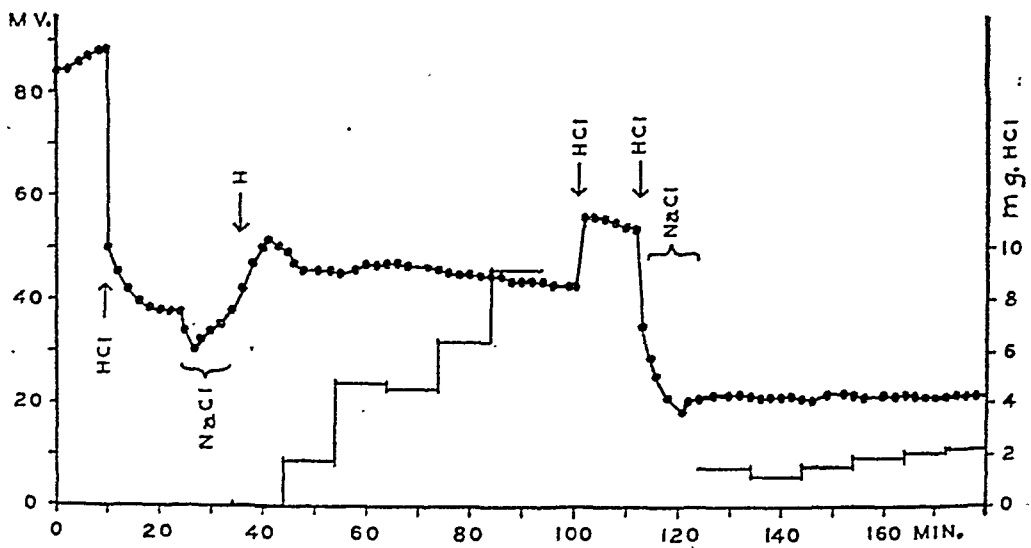

Fig. 4. At first HCl arrow, HCl of a pH of 0.77 was run into the chamber; this solution was washed out with saline as indicated by the bracket. At the time indicated by the *H* arrow, histamine was started (1 mgm. of diphosphate every 10 minutes for the duration of the experiment). At the second HCl arrow, HCl of a pH of 0.75 was placed in chamber; at the third HCl arrow, HCl of a pH of 0.5 was placed in chamber. This latter solution was washed out with saline as indicated by second bracket.

one minute after the flow had ceased is evidence that the increase in potential was not due to a flow potential. A total of eleven experiments was performed and they are summarized in table 1. The difference in the magnitude of the potentials of the resting and secreting stomach is given for each of these experiments in table 1. It can be seen that the increase in potential after application of the HCl was always definitely less than the difference between the magnitude of the potentials of the resting and secreting stomach. The rates of HCl secretions are also given in this table, and it can be seen that there is a fairly good correlation between the rate of secretion of HCl and the magnitude of the increase in potential after application of the HCl solution.

After reaching a maximum the potential in the majority of the experiments started to fall while the HCl remained in the chamber. In some experiments the potential showed a much more marked fall (fig. 3A) than in other experiments (fig. 3B). Replacement of the HCl by saline was always followed by a

further decrease to a value below the original pre-HCl level. In some experiments (fig. 3B) there was a comparatively rapid return to the level of potential that existed before the introduction of the HCl into the chamber. In other cases the potential remained below the original pre-HCl level for the duration of the experiment (1 to 2 hrs.). The chamber was always washed with large quantities of saline after removal of the HCl solution.

Effect of HCl solutions on the resting potential. Experiments were performed to determine the effect of HCl solutions on the magnitude of the resting potential. In every case there was a marked decrease in the magnitude of the potential following the application of HCl solutions of a pH range of from 0.64 to 1.1 (see fig. 4). In the fifteen experiments the potentials after HCl application were within 20 mv. of the potential after subsequent histamine stimulation, and in all but two experiments the potential was higher after HCl application than after subsequent histamine stimulation. After the HCl was replaced with saline the potential of the resting stomach gradually increased (over a period of about 1 hr.) to the original pre-HCl level (in the absence of further experimental procedures).

TABLE 1

Effect of HCl solutions on the potential of the secreting stomach

Experiments arranged with reference to secretory rates. Values in column 5 obtained by subtracting values in column 3 from those of column 4. Column 6 gives the difference in potential between resting and secreting stomach. S.R. refers to the secretory rate in milligrams of HCl per 10 minutes. Potentials in millivolts.

DOG	pH OF HCl	POT. BEFORE HCl	MAX. POT. AFTER HCl	Δ POT.	Δ POT. AFTER HIST.	S.R.
1	0.95	41.5	45.5	+4	30	1.3
2	0.64	40.0	44.5	+4.5	38	2.0
3	0.80	45	43	−2	40	2.1
2	0.75	35	39	+4	38	2.6
4	0.76	41	47.5	+6.5	39	3.5
5	0.75	37	43	+6	33.5	4.0
6	0.82	37.5	46.5	+9	32.5	4.2
7	0.75	43	56	+13	44.5	9.1
8	0.65	43.5	56.5	+13	33	9.9
8	1.03	44	56.5	+12.5	33	11.0
8	0.75	43.5	59	+15.5	33	12.4

Effect of histamine on the potential after it has been depressed by HCl. Figure 4 shows a typical experiment in which the effect of histamine on the potential and secretion was determined after the resting potential had been depressed by application of HCl. It can be seen that there was a comparatively small change in the magnitude of the potential with the onset of secretion and again (see fig. 2) the potential remained fairly constant while the secretory rate continued to increase. Two similar experiments were performed with essentially the same results.

Effect of HCl solutions on the relationship between secretion and potential. Since

the potential is often depressed in the secreting stomach following the introduction of HCl and replacement with saline, it would be of interest to determine the correlation between the potential and secretory rate under these conditions. In figure 3A it can be seen that a marked depression of the secretory rate is associated with the decrease in the potential and that as the potential increased the secretory rate showed a concomitant increase. In figure 3B the potential had returned to its pre-HCl level within 10 minutes after replacement of the HCl with saline. The secretory rate during the first 10 minute interval after the potential had returned to its HCl level was 80 per cent of its value before application of HCl. Figure 4 shows an experiment in which an attempt was made to depress the potential (which showed relatively little change while an HCl solution of a pH of 0.75 was in the chamber) by replacing the HCl solution with a

TABLE 2

Effect of HCl on the potential and secretory rate of the secreting stomach

Columns 7 and 8 give the percentage change in the potential and secretory rate before and after HCl

DOG	POT. BEFORE HCl	S.R. BEFORE HCl	pH OF HCl	POT. AFTER HCl	S.R. AFTER HCl	% Δ POT.	% Δ S.R.
2	40	2.0	0.64	45	1.3	+12.5	−35
6	37.5	4.2	0.82	39	2.5	+4.0	−40
2	35	2.6	0.75	36	2.1	+2.9	−19
8	44	11.0	1.03	44	12.4	0.0	+13
8	44	12.4	0.75	43.5	9.9	−1.1	−20
3	45	2.1	0.80	43.5	1.55	−3.3	−26
1	41.5	1.3	0.95	38.5	1.1	−7.2	−15
7	43	9.1	0.75*	21	1.5	−51.0	−84
8	43.5	9.9	0.65	19	0.1	−56.0	−99
5	37	4.0	0.75	14	0.3	−62	−92
4	41	3.5	0.77	6	0.2	−85	−94

* Replaced with HCl pH 0.50 before saline placed in chamber. HCl pH 0.50 remained in chamber approximately 2 minutes.

relatively concentrated HCl solution. This latter solution was washed out with saline after it had remained in the chamber for approximately 2 minutes. Following replacement with saline the potential decreased to a level around 21 mv. and remained at this level for the duration of the experiment (80 min.). The secretory rate was also depressed and remained so for the duration of the experiment. It should be pointed out that no attempt was made to measure the secretory rate for at least 10 minutes after the HCl had been replaced with saline. The chamber was always washed out with at least 900 ml. of saline following the application of HCl. In the resting stomach it was found that after this procedure the fluid in the chamber remained at a pH above 5.0. The data on the effect of HCl on the relationship between secretion and potential are summarized in table 2. The secretory rate given in column 6 is the secretory rate for the first period during which it was measured following replacement of HCl with saline.

The potential given in column 5 is the average potential for this same period. It can be seen that a marked decrease in potential is associated with a marked reduction in the secretory rate.

DISCUSSION. *Correlation of the present findings with those of other workers.* The experiments in this paper provide a basis for a tentative explanation of some of the apparently conflicting data cited in the introduction. The results of the experiments on the effect of histamine on the resting potential are strikingly similar to those of Mislowitzer and Silver (9). The finding that the secretory rate after histamine stimulation may increase after the potential has reached a relatively constant level and the fact that there is a relatively small change in the potential after histamine stimulation if the potential has been previously depressed by application of HCl, suggest an explanation for the findings of Quigley et al. (10). These investigators used pouch dogs and it is possible that the pouches possessed a relatively high basal secretory rate or that the pH of the fluid in the pouch was relatively low before experimental gastric stimulation and, therefore, administration of gastric stimulants would not be expected to be followed by a significant change in the potential. There is of course the possibility that in unanesthetized dogs the potential of the resting stomach in contact with a solution of relatively low acidity is of approximtely the same magnitude as in the secreting stomach. Obviously more work is needed on this point. Sarre's findings (14) are the most difficult to interpret. His finding following histamine of an increase in the negativity of the mucosal electrode with respect to an electrode on the thigh is probably equivalent to an increase in the magnitude of the potential across the stomach. This is similar to the relationship found in some of the experiments between secretory rate and potential (see fig. 3A) after HCl is placed in contact with the secreting stomach and then replaced with saline. This suggests that for some unknown reason the gastric potential in Sarre's experiments might have been originally depressed below the level at which active secretion occurs. Although Swyngedauw (15) found that in humans a change in the gastric potential was associated with the stimulation of gastric secretion, it is not clear from the description of his results in which direction the potential changed.

Factors that may play a rôle in the decrease of the potential after histamine. The HCl of the gastric secretion in contact with the saline in the chamber would be expected to give rise to a diffusion potential oriented toward the mucosal electrode. Introduction of an HCl solution of a concentration equal to that of the gastric secretion was never followed by an increase in the magnitude of the potential by as much as a half of the decrease in the magnitude of the potential after histamine stimulation. The fact that the increase in the potential after HCl was found to be definitely less than the magnitude of the diffusion potential between the gastric secretion and saline (found to be around 35 mv. (unpublished experiments)) might be interpreted as demonstrating that the increase in the potential after HCl is not a measure of the effect of the diffusion potential on the gastric potential. However, to assume that the formation of this diffusion potential would cause a decrease in the gastric potential of the above magnitude

implies that there is a continuous interface between the saline and the gastric secretion. If only a small portion of the stomach were secreting HCl then the diffusion potential, on the basis of the laws of electrical networks, would be expected to have a relatively small effect on the gastric potential, and as the area of secretion increased and as it approached the total area of that portion of the stomach in the chamber, the effect of the diffusion potential would be expected to increase and approach the magnitude of the diffusion potential at the boundary of the two solutions. It is significant that a fairly good correlation was found to exist between the magnitude of the secretory rate and the magnitude of the increase in the potential after HCl application (see experiments in table 1 in which the concentrations of the HCl solutions varied by only 0.07 of a pH, i.e., pH 0.75 to 0.82). In the experiments during which there was a high secretory rate the potential did not fall off very rapidly after introduction of an HCl solution. In the light of these observations it may be tentatively concluded that the initial increase in potential is a fair index of the effect of the diffusion potential on the gastric potential. The fact that HCl solutions, comparable in concentration to that of gastric secretion, depress the potential of the resting stomach suggests that the secretion of HCl by the stomach itself would result in a depression of the potential, and that this factor, together with the effect of the diffusion potential, may account for the decrease in potential after histamine. This conclusion would not be ruled out by the phosphate buffer experiments since it is reasonable to assume that a thin layer of HCl would be present at the surface of the secreting mucosa, and since the diffusion potential between the phosphate buffer and the gastric secretion has essentially the same magnitude as that between the gastric secretion and saline (unpublished experiments). On the other hand, consideration of the many possible factors that might influence the potential would make it premature to conclude that the above two factors alone account for the decrease in potential after histamine.

The relationship between secretion and potential. An examination of the data presented in this paper reveals that the relationship between the gastric potential and secretion is a complex one. The conclusion of Sarre (14) and of Quigley et al. (10) that the potential plays no rôle in secretion seems unjustified in the light of the evidence presented in this paper. The fact that a marked depression of the potential below the secretory level is associated with a marked decrease in the secretory rate is evidence in favor of the hypothesis that the potential plays a rôle in secretion. Obviously more work should be done with other agents that depress the potential of the secreting stomach. On the other hand, one might assume that the initial decrease in potential after histamine stimulation would indicate that there is less electrical energy available in the secreting than in the resting stomach. However, the exact opposite may be true since a consideration of the properties of electrical networks reveals that a decrease in the potential across the stomach might be associated with a marked increase in the electrical energy output inside the stomach, i.e., a decrease in the resistance of a shunt inside the stomach perpendicular to the surface would result in an increase in the current flow inside the stomach and a decrease in the IR

drop across the stomach wall. The fact that there is a decrease in the potential of the resting stomach after application of HCl solutions raises the interesting question as to whether energy has to be expended under these conditions to prevent HCl from penetrating the mucosa.

Nothing has been said in the above discussion about mechanisms that might be responsible for the modification of the potential at the interface between the mucosa and the external fluid. In the writer's opinion more information is needed concerning such things as the site of HCl formation (see Hoerr and Bensley (3), Hollander (4), and Henning (2)), the permeability of the resting and secreting stomach, the changes in resistance of the stomach after histamine stimulation, and the mechanism of the production of the gastric potential (see Lung (6), Marsh (8) and Korr (5) for evidence that the chemical potential energy of cells may manifest itself as electrical potential) before a discussion of these mechanisms would be profitable.

SUMMARY

A method is described for the measurement of the gastric potential and the rate of secretion of hydrogen ions from the same portion of the stomach. It was found that the potential of the non-secreting stomach, when saline was in contact with the mucosa, was between 70 and 95 mv. Histamine stimulation was followed by a decrease in the magnitude of the potential. The potential after reaching a new level remained relatively constant while the secretory rate continued to increase.

In an attempt to determine the effect of the diffusion potential between the gastric secretion and the saline on the gastric potential, HCl solutions of a pH range of that of the gastric secretion were placed in contact with the mucosa and the effect on the potential was determined. The increase in potential following this procedure was always less than the magnitude of the original decrease after saline. Application of HCl solutions of the above pH range to the resting stomach was followed by a decrease of the potential. After the HCl was replaced by saline the potential of the resting stomach gradually increased to approximately its original level. Histamine stimulation, while the potential was still depressed, was followed by secretion and comparatively little change in the magnitude of the potential. With a phosphate buffer in contact with the mucosa histamine stimulation was followed by a typical decrease in the potential. The rôle of these factors in the decrease of the potential after histamine stimulation is discussed.

It was found that application of HCl solutions to the secreting stomach and subsequent replacement with saline resulted in some experiments in a marked decrease in the potential. It was found that this decrease in the potential was associated with a decrease in the secretory rate.

On the basis of the results obtained in these experiments an attempt has been made to reconcile the conflicting reports in the literature on the relationship between gastric secretion and potential.

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EXPERIMENTAL OBESITY IN THE DOG

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Received for publication February 4, 1944

Experimenters in the field of obesity are aware of the controversy which still exists concerning the relative importance of lesions of the hypophysis and of the hypothalamus as causes of experimental obesity. Fröhlich (1) and Crowe, Cushing and Homans (2) are among those who attributed to lesions of the hypophysis a dominant rôle. Aschner (3), Erdheim (4), Bailey and Bremer (5), Camus and Roussy (6), Smith (7), Grafe and Grünthal (8), Economo (9) and others have declared that lesions of the hypothalamus alone may give rise to obesity. In the opinion of the authors, analysis of the literature yields evidence which supports the interpretation that either a lesion of the hypophysis or of the hypothalamus or both together may lead to obesity. Little or no explanation as to how such lesions may cause obesity has been offered.

In this paper observations on the effect of removal of the hypophysis and of various lesions of the hypothalamus on fat storage in the dog are presented. It has been found that either a removal of the hypophysis or the production of a lesion of the hypothalamus resulting in partial or complete destruction or denervation of the paired paraventricular nuclei may cause obesity. The additional bilateral destruction or denervation of the supraoptic nucleus enhances the rate of development of the obesity. The maximum degree of obesity follows a properly placed hypothalamic lesion without removal of the hypophysis. Following such a hypothalamic lesion there occur a loss and degeneration of the basophil cells of the hypophysis. The adrenal cortex does not atrophy; indeed, the animal becomes in a manner sensitized to this hormone, thereby favoring gluconeogenesis. This is believed to be significant in the pathogenesis of obesity through an effect on hunger and on fat formation and storage. The depression of thyroid and gonadic function which follows removal of the hypophysis or degeneration of its basophil cells also in itself leads to obesity at a slow rate, particularly when the dogs so modified are caged and given an ample food supply. A rapid gain in weight is invariably accompanied by a large food intake. It is concluded that the development of obesity is an expression of an excess of food intake over food requirements and not of any abnormal metabolic processes. With carbohydrate available, fat formation and storage are facilitated.

MATERIALS AND METHODS. Mongrel dogs were used as experimental animals. Most of them were kept in metabolism cages from one to three months to establish their weight and to determine their normal daily urine output. They were fed measured limited amounts of dog chow and horse meat except when for

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[2] On leave of absence for military duty.

experimental purposes unlimited but measured quantities were allowed. A few normal dogs were kept in cages for 6 months to 2 years under the standard conditions noted to serve as controls, while the remainder were subjected to various operative procedures. At least 10 to 20 animals of each of the different groups, proven so by thorough examination of serially cut microscopic sections of essential tissues were available for study. In this paper, to conserve space, the results on only a few of each class will be presented. The results on the others of the classes conformed with those reported on.

The required operations on the hypophysis and the hypothalamus were carried out through the oral approach under nembutal anesthesia and designed so as to yield the following groups:

1. Simple hypophysectomy dogs; those in which the entire glandular hypophysis, the stalk and the posterior lobe of the neural hypophysis were removed. The median eminence was not appreciably, if at all, injured.

2. Total hypophysectomy dogs; those in which the entire glandular and neural divisions of the hypophysis were removed, i.e., the median eminence was included.

3. Puncture dogs; these were subdivided into three groups. *A*. Those in which the stalk was severed, care being taken to disturb the hypophysis as little as possible, and then with a small scoop the median eminence was removed and a puncture wound made into the posterior hypothalamus to sever the fibers passing caudally from the paired paraventricular nuclei.

B. Those in which after severing the stalk and removing the median eminence the puncture wound was made rostrally in the central portion of the anterior hypothalamus.

C. Those in which after severing the stalk a puncture wound only was made in the mid-posterior hypothalamus deep enough to interrupt the fibers coming from the caudal portion of the paired paraventricular nuclei.

The dogs in the various classes were then observed for periods varying from 5 to 30 months. They were kept under dietary conditions similar to those used before operation. Periodically, both before and after operation, unlimited amounts of dog chow and meat were allowed for a period of a week and the amount of food eaten measured. It was hoped that the amounts consumed before and after operation would serve as a measure of the dog's hunger or state of satiation. Daily urine measurements were made and weekly records kept. Periodically the blood glucose and the blood cholesterol levels were determined. Insulin and sugar tolerance determinations also were made. After the period of observation the dogs were sacrificed and completely autopsied. On a limited number of dogs from each of the classes liver glycogen and liver fatty acid determinations were made in the postabsorptive stage (16 to 24 hrs. after a last meal) at the time of sacrifice.

At autopsy the brain was perfused in situ with 10 per cent formalin and then fixed in this solution for several days. Then the hypothalamus was removed, serially sectioned at 20 microns and every third slide stained with cresyl violet. The sella and its contents were similarly sectioned at 20 microns and stained with

hematoxylin and eosin. The sellar contents from many of the dogs of group 3 were separated carefully from the bone after fixation to eliminate the necessity of using decalcifying agents and to permit staining of the hypophysial tissue for differentiation of cell types according to the method of Rasmussen. Such tissue was serially sectioned at 5 microns.

Among the other organs and tissues studied for this research were the thyroid, the pancreas, the liver, the adrenals and the gonads. Care was taken to obtain sections from 5 to 7 different areas of the pancreas. These were stained with

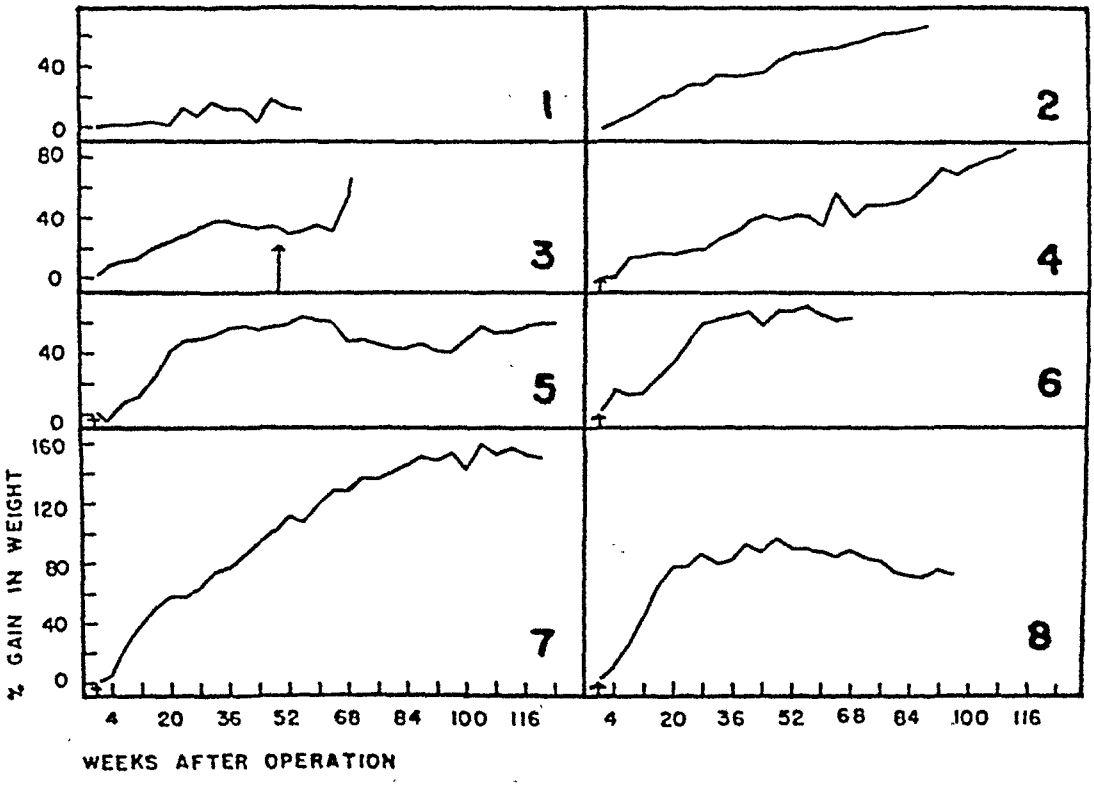

Fig. 1. Plots of percentage gain in weight against time after caging or caging and operation. 1 and 2, normal dogs; 3, normal for 6 months and then simply hypophysectomized; 4, a simple hypophysectomy dog; 5 and 6, total hypophysectomy dogs; 7 and 8, puncture dogs.

hematoxylin and eosin and according to the method of Masson and the Mallory-azan technique.

Results. *Analysis of the body weight and urine charts.* Normal dogs kept under the conditions outlined above vary in their response as regards body weight. Certain breeds which are known to have a tendency to gain weight with age may in 2 years of observation exhibit a definite but slowly developing weight gain of from 30 to 65 per cent, while other types may show only a slight weight increase from 0 to 10 per cent (fig. 1). It has been learned from our experiences in attempting to establish a base line for normal dogs for comparison with dogs modified by operative procedures that ideally, pure bred dogs, of a breed which does not normally exhibit a definite tendency to obesity, should be chosen for an investigation of experimental obesity.

With increasing obesity normal dogs exhibit no alteration in urine output (fig. 2).

Simple hypophysectomy dogs exhibit a definite tendency to obesity. The development of their obesity is slow but definitely more rapid than that which occurs in the normal dog with spontaneously developing obesity. Such dogs may exhibit an increase of 30 to 85 per cent over their original weight after 2 years of observation (fig. 1). The weight curves of simple hypophysectomy dogs indicate that conclusions as to the effect of the procedure on obesity must not be drawn before an adequate period of time has elapsed. In healthy animals after long periods of observation, i.e., 1½ to 2 years or more, the degree of weight

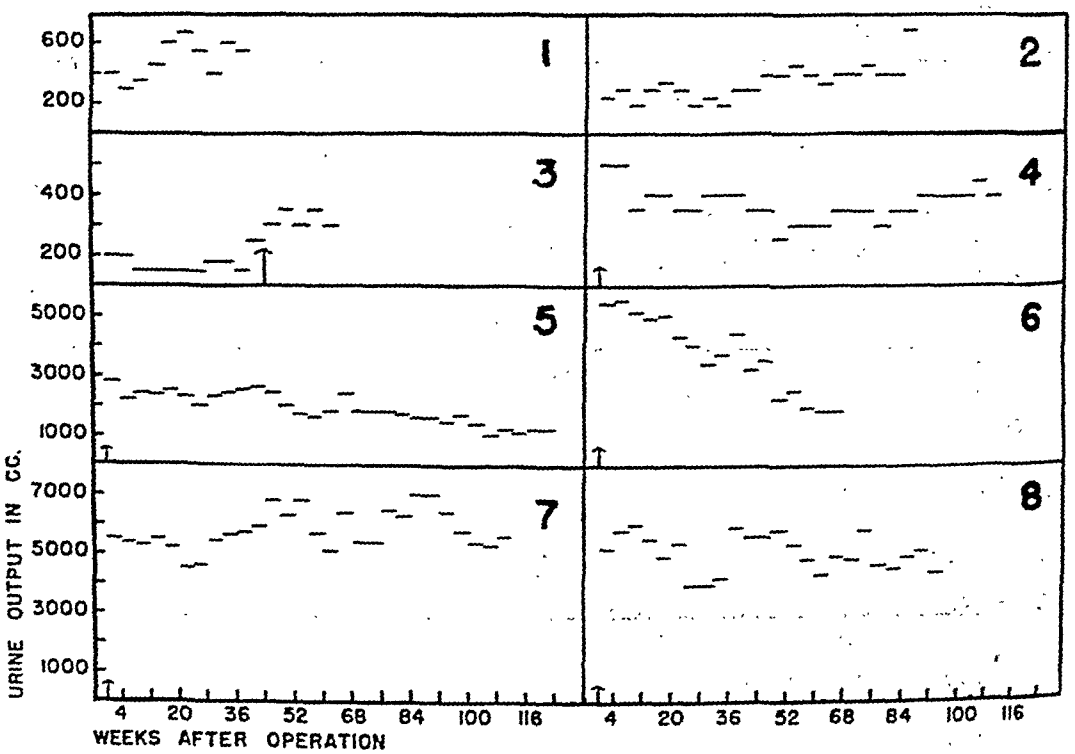

Fig. 2. Plots of urine output against time after caging or after caging and operation of dogs whose weights are shown in figure 1.

increase is from 20 to 30 per cent greater in simple hypophysectomy dogs than in normal dogs with a definite tendency to obesity.

The urine output of this class of dogs is normal, or normal with an occasional slight increase over the normal (fig. 2).

Total hypophysectomy dogs exhibit a much more rapidly developing obesity. The degree of weight increase in the first year after operation is usually definitely greater than that of the simple hypophysectomy dog but after 2 or more years of observation the 2 classes exhibit very similar weight increases (fig. 1).

Total hypophysectomy dogs exhibit a permanent and marked diabetes insipidus.

Puncture dogs of the group A class exhibit the most rapidly developing and

the greatest degree of experimental obesity. After 2 or more years of observation the weight increase may reach 150 per cent above the preoperative level (fig. 1). The degree of increase is greater than that exhibited by the other classes of dogs. Such dogs are modified by the operation in a manner which results during the period of rapidly developing obesity in a greater food intake than in any of the other classes of dogs.

Puncture dogs exhibit a permanent maximum diabetes insipidus (fig. 2).

Group B dogs exhibit only a slowly developing moderate degree of obesity. It has not been possible to produce a lesion which leads to a state of *marked diabetes insipidus* without the development of any obesity. From the clinical literature it is evident that in man such a condition is possible. It is felt that in the dog complete bilateral destruction of the supra-optic nucleus alone would effect such a result but this can not be accomplished by our method of operation. Always in our dogs with destruction or denervation of the supraoptic nuclei the median eminence is removed and this is associated with a mechanical loss or a retrograde degeneration of part of the cells of the rostral portion of the paraventricular nuclei.

Group C dogs exhibit a rapid, marked increase in weight. The amount of weight increase is slightly less in our experience than when the entire supraoptico-hypophysial system is destroyed. From an analysis of our data the impression is gained that complete or marked loss of pitressin aids in the development of obesity. It is realized that the degree of difference in weight increase between dogs of the A and C groups of this class of dogs might have been found to be within the range of variation for those of group A if the number of dogs operated on had been larger. There exists, however, a possible anatomical basis for a difference between these two groups of dogs. Thus the destruction or denervation of the paired paraventricular nuclei which results in obesity is partially effected in total hypophysectomy dogs because, as before stated, destruction of the median eminence results in a marked loss of cells from the rostral portion of this nucleus. This cell loss conceivably may be of significance in effecting those changes in the body which lead to the obesity which is evident in greater degree on destruction of the major portion of the entire paraventricular nucleus through a posterior hypothalamic puncture.

Anatomical studies. Microscopic examination of the hypophysis and the hypothalamus from 2 normal dogs which became markedly obese showed both to be cytologically normal (fig. 3). Microscopic examination of the adrenals, the gonads, the liver and the pancreas reveals nothing obviously abnormal. The thyroid gland of one showed low acinar epithelium with inspissated colloid, that of the other showed large but vacuolated cells without colloid formation. Such a finding has been reported for the hibernating hedgehog by Peiser (10). The distribution of the accumulated fat was similar to that found in dogs which became obese after the operative procedures.

Examination of the hypothalamus after simple hypophysectomy reveals an 80 to 85 per cent loss of the cells of the supraoptic nuclei. The paraventricular nuclei are practically unaffected (fig. 4).

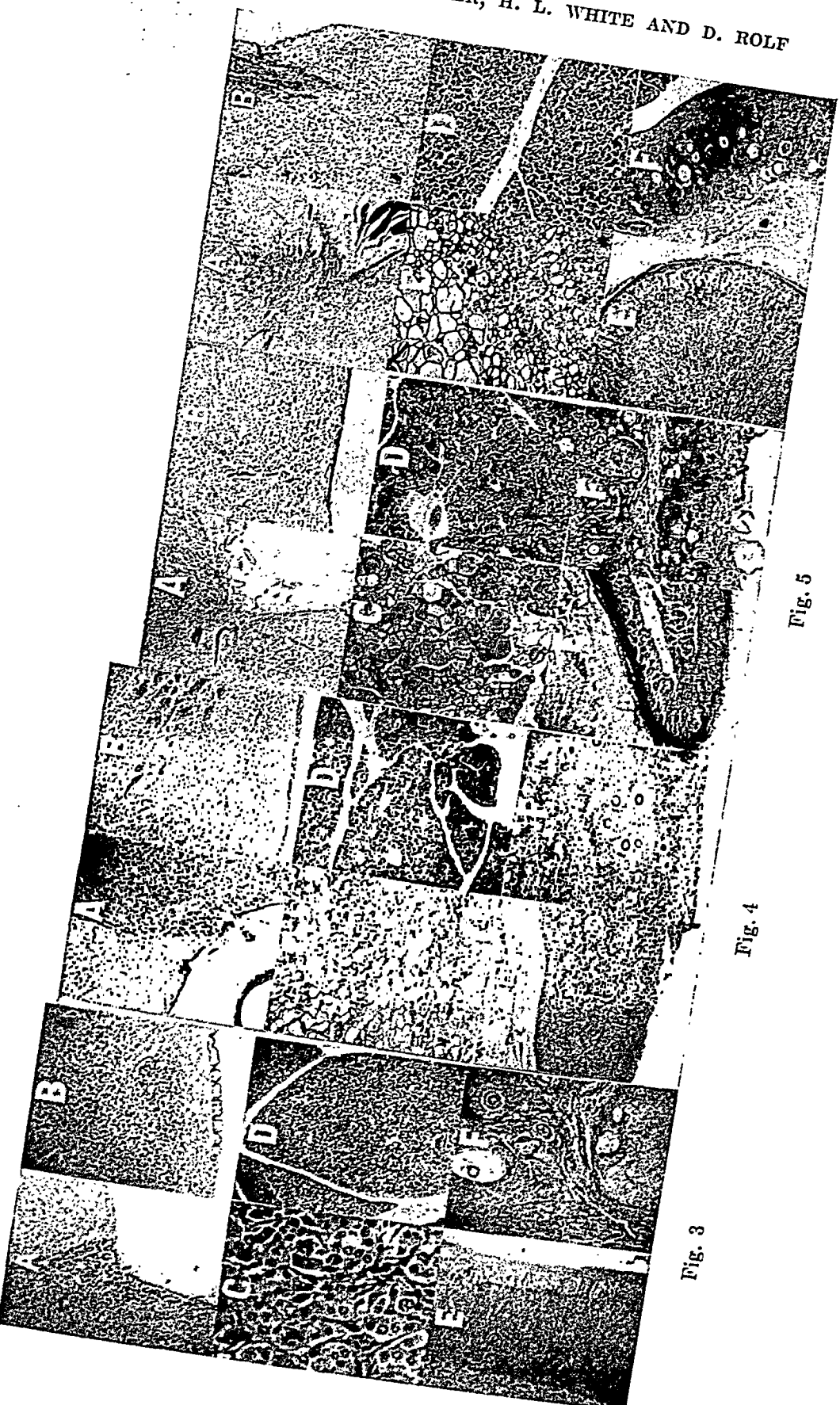

Fig. 3

Fig. 4

Fig. 5

Fig. 9

Total hypophysectomy, i.e., median eminence included, results in a complete loss of the cells of the supraoptic nucleus and a 30 to 50 per cent loss of the cells of the rostral portion of the paraventricular nucleus. No other nuclei or hypothalamic areas are appreciably affected (fig. 5).

Simple and total hypophysectomy results in changes in other tissues and endocrine glands which are of significance in our problem (figs. 4 and 5). The fat storage which results occurs in the subcutaneous tissue of the neck, the trunk, the abdominal wall and the extremities. There is an increase of fat in the pericardium, in the omentum, in the retroperitoneal areas, particularly in the perirenal regions. There is no obvious structural difference in the fatty tissues grossly or microscopically, from similar fatty tissues of the normal dog.

To determine the hypothalamic lesion responsible for fat storage lesions were made in more than 200 dogs. At various intervals after the operation the dogs were sacrificed and the hypothalami serially sectioned and stained for cells with cresyl violet. It can be stated from a correlation between the histological lesion and the effect on body weight that lesions of the anterior hypothalamus alone do not cause obesity. Lesions of the posterior hypothalamus do. From observations such as are depicted in figure 6 on dogs 9 and 10 it was suspected that the bilateral destruction or degeneration of the paraventricular nucleus was the essential lesion.

In each of these 2 dogs a lesion, caudal to the region of the paraventricular nuclei was made in the posterior hypothalamus. The pars distalis and posterior lobe also were removed in both, but the median eminence was not disturbed appreciably. These 2 dogs were chosen because of a critical difference in their

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Fig. 3. Photomicrographs of tissues from dog 2, a normal dog which became obese. A. supraoptic nucleus × 45; B. paraventricular nucleus × 45; C. thyroid gland × 610. Note the absence of colloid, cytoplasm vesicular. D. pancreas × 45; E. adrenal × 45; F. ovary × 45.

Fig. 4. Photomicrographs of tissues from simple hypophysectomy dog 4. A, supraoptic nuclei ×45; at least 20 per cent of the cells remain. B, paraventricular nucleus × 45, caudal portion. C, thyroid gland × 45. Note small acinar, inspissated colloid, many acini without colloid. D, pancreas × 45 showing hyperplasia and hypertrophy of islets. E, adrenal ×45 showing marked atrophy of the zona fasciculata and of the zona retucilaria. The zona glomerulosa is of normal width but the nuclei are pale staining, the cytoplasm vesicular. The medullary tissue also is atrophied. F, ovary × 45. Note the atretic follicles, absence of mature follicles.

Fig. 5. Photomicrographs of tissues from total hypophysectomy dog 5. A, supraoptic nucleus × 45. Note absence of cells. B, paraventricular nucleus × 45, caudal portion; at least 80 to 85 per cent of the cells present. C, thyroid × 45. Note flat acinar epithelium, many acini without colloid. D, pancreas × 45. E, adrenal × 45. Note marked atrophy of all zones except the glomerular. The cells in this show pale staining nuclei, the cytoplasm is vesicular and the medulla is atrophied. F, ovary × 45. Note only immature follicles.

Fig. 9. Photomicrographs of tissues from dog 8, of the puncture type. A, supraoptic nucleus × 22. Note absence of cells. B, paraventricular nucleus × 22, caudal portion. Note absence of cells. C, thyroid × 22. D, pancreas × 22. The islets are small, the cells show degenerative changes. E, adrenal × 22. Note the normality of the zones. F, ovary × 22. Note absence of mature follicles.

lesions. In one, which became obese, the cells in the caudal portions of the paraventricular nuclei are absent (retrograde degeneration); in the animal which did not become obese the nucleus has its normal length (fig. 7). The shortening of the paraventricular nuclei in the obese dog is due to retrograde degeneration of the cells following the interruption of their axons by the posterior hypothalamic puncture. The puncture in the other dog did not sever these axons. The paraventricular nuclei give rise to fibers going to the neural divisions of the hypophysis and to fibers which descend down the brain stem.

After this observation all our material was rechecked and a definite correlation between the degree of obesity and the degree of bilateral destruction or cell loss of the paraventricular nuclei established.

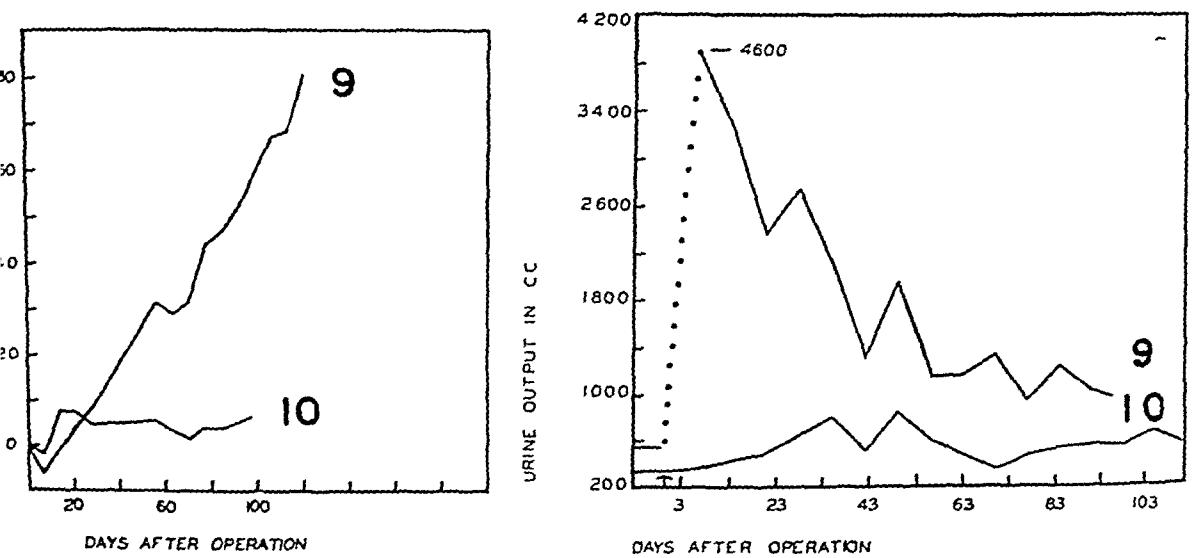

Fig. 6. Plots of percentage increase in weight against time and of urine output in days after operation for dogs 9 and 10.

The cell loss of most significance in causing obesity is from the caudal portion of the nucleus and must be bilateral. The rostral portion of the paraventricular nuclei innervates principally the median eminence and these cells probably innervate mostly or only pituicytes (Heinbecker and White, 11). The maximum degree of obesity results when the entire paraventricular nucleus disappears, partial loss results in only moderate obesity. Bilateral destruction of the lateral mammillary and of the mammillary nuclei does not lead to obesity.

Support for the above conclusion also has been found in the histological studies of the hypothalami of 4 cases of Cushing's syndrome in all of which obesity was present. In this material (Heinbecker, 12) the essential and sometimes only lesion present is a marked loss of cells in the paired paraventricular nuclei.

In the hypophysectomized dog the thyroid glands (figs. 4 and 5) are reduced in size. Microscopically the amount of colloid is greatly reduced and often markedly inspissated. The acini are small, many of them being without a lumen. Often large acidophilic cells are seen within and also apparently outside the acini. There is a considerable increase in fibrous tissue and in lymphoid infiltration.

The gland appears to lack the stimulus for the formation and the release of colloid.

The adrenal glands (figs. 4 and 5) are greatly diminished in size. Microscopically they show a marked increase in the amount of fibrous tissue in the capsule of the gland. There is a diminution in the width of the cortex. In dogs autopsied 2 years or more after hypophysectomy the fascicular and reticular layers may be almost completely atrophic, with the glomerular layer even somewhat taller than in the normal gland but the cells paler staining and more

Fig. 7

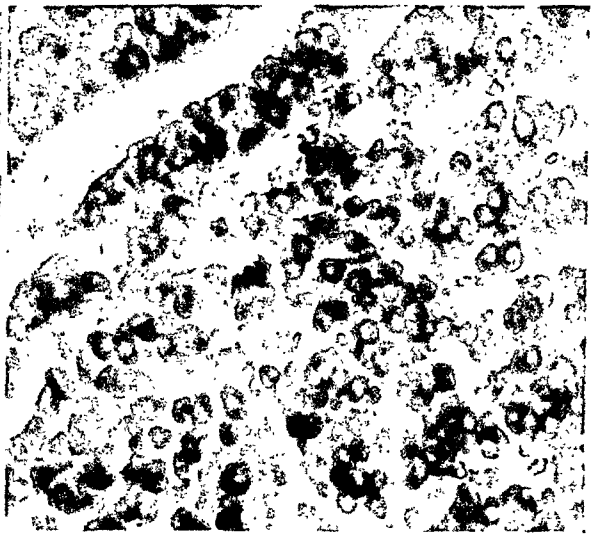

Fig. 8

Fig. 7. Photomicrographs 12.5 diameters of frontal sections through the hypothalami of two dogs 9 and 10. Note in dog 9 the paraventricular nucleus has ended at a level still in the optic chiasma and more rostral than the section shown from dog 10, caudal to the chiasma, in which the nucleus is still present.

Fig. 8. Photomicrograph × 340 from anterior lobe of hypophysis of dog 8 showing eosinophils and chromophobes with no basophils remaining.

vesicular. After this longer period the medulla, too, becomes appreciably altered. It is atrophied, with the remaining cells showing marked degenerative changes. Earlier, i.e., 6 to 12 months after hypophysectomy, the cortex may simply be narrowed in all its layers while the medulla may show little obvious change.

In the female the ovaries (figs. 4 and 5), the breasts and the uterus show marked gross atrophy. Histologically there is seen a failure of the follicles to mature. In the dogs sacrificed after 2 years the interstitial tissue is atrophic, a condition not apparent in the dogs sacrificed early. In the male the testes are atrophied to one-third or less of their normal size. On microscopic examination

there is marked atrophy of the glandular elements with a failure of spermatogenesis. In the dogs sacrificed 2 years or more after operation the interstitial tissue also is obviously diminished in amount.

The pancreas (figs. 4 and 5) grossly show variable changes. On microscopic examination the acinar tissue is normal. The islets in some show definite hypertrophy and hyperplasia, in others there may be an atrophy with an apparent diminution in the number of the islets, in others no obvious deviation from the normal is apparent. It is the impression of the authors that the changes in the pancreas are probably the result of changes in other structures rather than a direct expression of the loss of the glandular hypophysis.

The liver in healthy operated dogs is grossly normal. Microscopically the liver cells are well preserved except when before sacrifice there has been some weight loss and a decrease in food intake when fatty infiltration may be marked. With increasing time after operation there is an increase in fibrous tissue throughout the liver.

Many of the above described effects of hypophysectomy have been noted also by others. They are described in order to compare the effect on similar structures of a hypothalamic puncture in the posterior hypothalamus so placed that degeneration of the paraventricular nuclei follows. The hypophysis is disturbed as little as possible but the stalk must be severed to approach the posterior hypothalamus by this route. In such a dog the size of the remaining glandular hypophysis may be almost normal or reduced by as much as 50 per cent, depending on the degree of trauma at operation. The hypophysis remaining is adequate to insure a normal insulin tolerance and a normal renal blood flow (White, Heinbecker and Rolf, 13).

Examination of the glandular hypophysis reveals in all instances an almost complete loss of the basophil cells, the few remaining ones being deprived of their granules (fig. 8). The eosinophils are relatively increased in number because of the loss of basophils. The possibility of an increase in eosinophils exists but no count to determine their actual number has been made. The chromophobes are not abnormal.

The gross and microscopic appearances of the thyroid, the pancreas and the liver are similar to those exhibited in these organs after simple or total hypophysectomy (figs. 4, 5 and 9). The ovaries show some atrophy and a suppression of the normal maturation of their follicles, the testes fail to show normal spermatogenesis but the interstitial tissue of the gonads appears normal. The greatest difference occurs in the adrenals. Here there is no gross or microscopic atrophy as occurs after hypophysectomy. Insufficient material is available to answer the question as to whether or not there is a hypertrophy. If there is, it is not sufficiently marked to be obvious.

The distribution of the fatty deposits is similar to that seen in extremely obese normal dogs or in obese dogs after hypophysectomy. The use of special stains has shown the abnormal deposits of fat in the skin to be composed of neutral fat and cholesterol (Liebermann-Burchardt reaction).

Functional studies. The blood glucose values (Somogyi method) after 16 to

24 hours of fasting for 15 normal dogs based on 2 to 8 observations for each dog varied from 80 to 65 mgm. per cent with an average value of 76 mgm. per cent. Values obtained for the fasting blood glucose of 8 simple hypophysectomy dogs with 2 to 8 observations on each dog varied from 79 to 50 mgm. per cent with an average value of 65 mgm. per cent. Similar observations on 6 total hypophysectomy dogs gave values from 67 to 55 mgm. per cent with an average of 60 mgm. per cent. For 7 puncture dogs the values ranged from 87 to 55 mgm. per cent with an average of 72 mgm. per cent. There is a greater fluctuation in the values for the puncture and simple hypophysectomy dogs than for normal dogs. Total hypophysectomy dogs have a somewhat lower blood glucose level than simple hypophysectomy dogs but the values also fluctuate markedly. Our observations confirm the fact established by others that loss of the glandular hypophysis results in a rapid fall in the blood glucose level with fasting. Puncture dogs on the other hand withstand fasting without the development of dangerously low hypoglycemic levels.

The insulin sensitivity of simple and total hypophysectomy dogs is greatly increased, that of the puncture dogs is unchanged.

The instability of the blood glucose levels in hypophysectomy and puncture dogs is regarded as a significant factor in causing such dogs to experience hunger. Hunger is manifested most clearly by the puncture dog.

Evidence of increased activity in the hypothalamus and other regions of the brain stem during the downward shift of the blood glucose level following the administration of insulin has been presented by Bartley and Heinbecker (14). According to Kabat et al. (15) stimulation of the hypothalamus results in inhibition of peristalsis of the stomach and of the small intestine. It would be reasonable to expect that on excitation of the brain stem of a dog in which the inhibitory region, i.e., the hypothalamus, had been functionally eliminated, there would occur increased peristalsis of the stomach and small intestine because activity of the exciting or parasympathetic system would be facilitated. The excitation of cells in the brain stem caused by a downward shift of blood glucose could also directly stimulate the cells through which the sensation of hunger is experienced. They, in turn, could be expected to activate the neural mechanism through which the peristalsis of the stomach and of the small intestine is increased as it is during hunger.

The cause of the rapid depression in blood glucose level in hypophysectomized dogs is to be found in their high tissue utilization of carbohydrate (Russell, 16), their insulin sensitivity and their depressed adrenal cortical function. The greater ability to utilize carbohydrate presumably would decrease the use of fat and permit its storage, particularly when the general metabolic demands are lessened because of a depression of thyroid and gonad function.

In puncture dogs the adrenal cortex does not atrophy. The ability of the blood sugar level to be maintained at a higher level on fasting than in hypophysectomized dogs is taken as evidence of functional capacity of the adrenal cortex. The evidence that puncture dogs are sensitized to exogenous adrenal cortical hormone in its influence on renal blood flow (Heinbecker, Rolf and White, 18)

cannot be regarded as final evidence that they are sensitized to it as a modifier of carbohydrate metabolism but it suggests the possibility.[3] Further evidence in support of the concept of exaggerated or unbalanced function of the adrenal cortical hormone has been furnished by McQuarrie et al. (18) in their study of cases of Cushing's syndrome. In such cases, some associated with an adrenal tumor and some without such an association, the blood sodium level has been found elevated, the blood potassium level depressed. In similar cases not due to an adrenal tumor the primary pathology has been found to be an atrophy of the paired paraventricular nuclei in the hypothalamus (Heinbecker, loc. cit.). The effect of the adrenal cortical hormone, increased because of its increased amount in tumor cases and because of a hormonal imbalance in the others, could be expected to increase gluconeogenesis and directly or indirectly to increase fat formation from glucose and aid in its storage. Evidence has been presented by Brobeck et al. (19) to show that in rats which became obese after a hypothalamic injury, the conversion of carbohydrate to fat is increased.

There is no experimental evidence to indicate how extracts of the adrenal cortex lead to increased fat storage. It may be that fat storage is increased because the greater amount of carbohydrate available through gluconeogenesis lessens the necessity for fat utilization and thereby leads to its storage. In puncture dogs the caloric requirements too are lessened because of a depression of thyroid and gonad functions resultant from a loss of basophil cells in the hypophysis. The circumstances are such that food intake is well maintained and food requirements are lessened; under them obesity would be expected to follow.

Liver glycogen values 16 to 24 hours after the last food intake were obtained in a small number of dogs. In 1 normal dog the liver glycogen was 9.12 mgm. per cent; in 1 simple hypophysectomy dog, 7.1 mgm. per cent; in 1 total hypophysectomy dog, 6 mgm. per cent; in 4 puncture dogs, 9.3 mgm. per cent, 5.4 mgm. per cent, 6.8 mgm. per cent and 5.8 mgm. per cent, respectively, the last being a 3 months-old puppy. The saponifiable liver fatty acid for the normal fat dog was 0.297 mgm. per cent; for the simple hypophysectomy dog, 5.35; for the total hypophysectomy dog, 4.62 mgm. per cent; for 3 of the puncture dogs, 3.73 mgm. per cent 4.6 mgm. per cent and 1.67 mgm. per cent, the last reading being for the 3 months-old puppy. The liver glycogen values indicate a high carbohydrate reserve, adequate to permit a minimum utilization of fat for metabolic needs. The livers did not show fatty infiltration on microscopic examination.

Blood cholesterol values (modified Bloor method) for 13 normal dogs averaged 140 mgm. per cent; for 3 simple hypophysectomy dogs, 223 mgm. per cent; for 5 total hypophysectomy dogs, 299 mgm. per cent; and for 10 puncture dogs, 194 mgm. per cent. From these results it appears that obesity in adult dogs is associated with an elevated blood cholesterol but there is no correlation between the degree of obesity and the degree of elevation of the blood cholesterol. The

[3] The tissues sensitized include the cells of the glandular hypothysis.

fatter puncture dog has a lower blood cholesterol than the fat total hypophysectomy dog.

It is of interest to note that hypophysial extract[4] lowered blood cholesterol in simply hypophysectomized fat dogs from an average value of 250 mgm. per cent to 110 mgm. per cent. It did not change the blood cholesterol of puncture or of normal dogs appreciably. Adrenal cortical extract (Upjohn[5]) given subcutaneously 4 to 8 cc. daily elevated the blood cholesterol of simply hypophysectomized fat dogs from an average value of 223 mgm. per cent to 315 mgm. per cent; of totally hypophysectomized fat dogs from an average value of 299 mgm. per cent to 460 mgm. per cent; of fat puncture dogs from an average value of 194 mgm. per cent to 315 mgm. per cent. This dosage did not alter the normal dog's blood cholesterol. Thyroid extract, 1/10 gram per kgm. of body weight per day for 14 days, lowered the blood cholesterol of fat puncture dogs from an average value of 194 mgm. per cent to 135 mgm. per cent; of normal dogs from an average value of 140 mgm. per cent to 80 mgm. per cent.

Observations were made to estimate the effect of the various operative procedures on food intake on 2 dogs in each class. The amount of food taken when given in unlimited amount for a week before operation was compared with that eaten at various periods after operation. The results indicate that under such circumstances both normal and operated animals eat large quantities of food but no striking difference was noted between the normal and the various operated classes after operation. Any 20 to 30 kgm. dog given unlimited food will eat between 1 to 2 kilos of meat plus 0.25 to 0.50 kilo of dog chow per day. During the period of rapid weight gain in operated dogs their appetite is particularly good, that of the puncture group always being striking. About 1 dog in 3 of the simple and total hypophysectomy groups and of the puncture groups after 1½ or more years of good health and weight gain will suffer a diminution in their water and food intake and become emaciated. At autopsy the precipitating cause invariably found has been a basilar meningitis particularly marked in the region of the hypothalamus. Such dogs show fatty degeneration of the liver. The islets of the pancreas frequently are markedly diminished in number and size. It is interesting to note that dogs previously exhibiting marked diabetes insipidus because of a proven loss of cells of the supraoptic nucleus nevertheless under such circumstances lose their thirst and have a normal or diminished urine output.

It is felt that our findings are consistent with the interpretation of Kabat and his associates (loc. cit.) that obesity in the rat develops on the basis of an increase in food intake but dogs do not exhibit the same degree of increase in hunger as do rats after appropriate hypothalamic lesions.

Pathogenesis of experimental obesity. Conditions which lead to a degree of obesity which surpasses that which occurs spontaneously in some normal dogs

[4] Preloban Niphanoid supplied through the generosity of the Winthrop Chemical Company.

[5] The adrenal cortical extract was supplied in part through the kindness of the Upjohn Company.

with caging or age, and in simple or total hypophysectomy dogs, exist in the puncture dog. Two essential features, the gross normality of the adrenal glands and the increased sensitivity to exogenous adrenal cortical hormone, set this class of dog apart and are considered responsible for the abnormal storage of fat. Long et al. (20) have shown that adrenal cortical hormone is capable of augmenting gluconeogenesis. The additional carbohydrate formed thereby would be expected to lead to a greater utilization of carbohydrate for necessary bodily requirements and to lessen the demand for fat. Under such circumstances any excess of ingested fat or fat formed from excess carbohydrate intake or formation would be available for storage.

Support for the concept that adrenal cortical hormone leads to fat storage is found in the work of Hewer (21) who showed that the administration of lipoid extract of the adrenals of beef to rats results in obesity. Similar evidence has been presented by McKinley and Fisher (22) and by others.

Clinical evidence also is available to indicate that adrenal cortical hormone is concerned in the development of obesity. Thus in cases of adrenogenital syndrome due to an adrenal tumor, abnormal obesity is characteristically present.

Additional factors which doubtless aid in the speed of development of obesity not only in puncture dogs but also in the simple and total hypophysectomy dogs are the depression of thyroid and gonad activity.

It has been found by Biedl (23) and ourselves (unpublished data) that total thyroidectomy in the dog leads slowly to a loss of weight, possibly in part because of a depression of appetite. In our experience the moderate depression of thyroid activity diminishes bodily requirements for nutritive material but permits a maintained food intake and therefore storage of fat can and is known to occur. It has been established in the human that the weight increase in hypothyroidism is not only due to fat but also to retained water. The depression of gonad activity likewise is known to lead to a depression of the basal metabolic rate of about 20 per cent (Loewy and Richter, 24). With a maintained food intake storage of fat is known to occur.

Because of the great increase in fat storage which can occur spontaneously in the apparently normal dog it seems unnecessary to postulate an abnormal mechanism for the formation and storage of fat in our altered dogs. No evidence for any such alteration in metabolism has been found, all differences being quantitative rather than qualitative. Genetic factors undoubtedly exist but the manner in which they exert their influence is unknown. In man the exaggerated influence of or absence of certain hormones seems to some degree to determine the locale of fat storage. For example, in Cushing's syndrome the fat storage occurs primarily in the face, neck and trunk whereas in hypogonadism it is chiefly about the pubis and the hips. No such differences have been observed in the various classes of dogs studied in this research.

Our results have indicated a more rapid development of obesity when the supraoptico-hypophysial system innervating the pitressin-forming tissue was totally or nearly totally inactivated; when total or nearly total diabetes insipidus exists. The appearance, activity and blood cholesterol values, especially of the

puncture dogs, do not indicate that the loss of pitressin lowers the metabolic rate. Possibly the explanation for the rapidity of their fattening is to be sought in evidence presented by Britton and Corey (25) and by others, which indicates that there is an antagonistic action between pitressin and the hormone from the adrenal cortex. It is conceivable therefore that the hormone of the adrenal cortex may become more effective when pitressin is lacking. Additional support for this concept is found in the clinical evidence that in Cushing's syndrome due to an adrenal cortical tumor, polyuria is frequent. In such a case the supraoptic and paraventricular nuclei have been shown to be histologically normal (Heinbecker, loc. cit.). Under such circumstances it is felt that the secretion from the adrenal tumor probably is responsible directly for the neutralization of the effect of pitressin in preventing polyuria. As stated above another possible explanation is to be found in our anatomical evidence that following removal of the median eminence there is a loss of cells, particularly in the rostral division of the paraventricular nuclei. Possibly some of the cells lost are of the class which normally send their fibers caudally to exert an influence similar to that exerted by other paraventricular cells in the caudal division of the nucleus, the loss of which ultimately causes the development of obesity. If, however, they are concerned only in activating pituicytes in the median eminence their influence on obesity would have to be considered indirect through an unbalanced increase in the effect of the adrenal cortical hormone.

It is deemed unlikely that the fibers going to the median eminence from the paraventricular nucleus cause the secretion of a hormone whose loss is responsible for the disappearance of basophil cells from the hypophysis of puncture dogs, because of the knowledge that marked diabetes inslpidus may exist in humans without obesity and without a suppression of ovarian function such as is associated with hyalinization of the basophil cells. Their presumed intactness in such cases would argue against the loss of pitressin being responsible for the hyalinization of the basophil cells. This is also borne out by the fact that stalk section, which results in considerable loss of pitressin, does not in itself lead to hyalinization of the basophil cells of the hypophysis.

It is of interest to note that the cells of the two nuclei, supraoptic and paraventricular, are of similar cytological character. Destruction of one of them, the supraoptic, leads to thirst and to polydipsia, destruction of the other, the paraventricular, to hunger and to polyphagia. The rostral division of the paraventricular may play a dual function. Some of the cells innervate, in part, the median eminence and therefore probably are concerned in the secretion of pitressin and thereby in the regulation of water balance while others as stated above may have an influence on obesity. The frequent accompaniment of pathological obesity with mild disturbances in water balance may find an explanation in such a dual function of the paraventricular nucleus.

There remains unanswered the question as to how the loss of the paraventricular nucleus leads to regressive changes in the gonads and to changes in carbohydrate metabolism and fat storage. These effects probably are initiated by changes in the effectiveness of the adrenal cortical hormone directly on carbo-

hydrate metabolism and indirectly through an influence on the basophil cells of the hypophysis. It is fairly certain that it is not through nervous pathways connected with the sympathetic or parasympathetic nervous system, because no such changes are effected by complete sympathectomy or by section of the vagus nerves beneath the diaphragm. It seems more probable that the fibers which pass caudally from the paraventricular nucleus innervate cells within the brain stem which secrete a hormone which directly or indirectly influences the adrenal cortex or the basophil cells of the hypophysis. It is suspected that this may be in the region of the epiphysis because clinical and experimental evidence, admittedly not conclusive, has pointed to this region of the nervous system as having an influence on fat storage and on the sex organs. Investigations are in progress to trace the destination of the fibers descending from the paraventricular nucleus.

SUMMARY

Experimental evidence is presented to show that obesity in the dog results from bilateral destruction or retrograde degeneration of the paired paraventricular nuclei, particularly of their caudal portions.

Marked obesity results when destruction or denervation of the neurohypophysis and destruction or retrograde degeneration of the paired caudal paraventricular nuclei co-exist.

Removal of the pars distalis itself in the adult dog results in a less marked and less rapid development of obesity but there does occur a slow weight increase which becomes marked with time.

The presence of the pars distalis in animals with the supraoptic and paraventricular nuclei destroyed or degenerated is favorable to the rapid development of marked obesity.

Destruction or denervation of the caudal portion of the paraventricular nuclei leads to changes in the body which increase the food intake. The organism and in particular the cells of the glandular hypophysis are rendered sensitive to the adrenal cortical hormone and probably because of this there results a marked loss of basophil cells in the glandular division of the hypophysis.

This results directly in changes in the thyroid and in the gonads and probably indirectly in the islet cells of the pancreas. The adrenals do not atrophy. The alterations in the metabolism effected by such changes result in the accumulation of fat in various tissues of the body. Total and simple hypophysectomy dogs show in addition to changes in the thyroid, the gonads and the pancreas an atrophy of the adrenal cortex. This may explain the fact that while they become obese the rate of accumulation of their fat and its amount are less than in the "puncture dogs."

Obesity is considered to result because of an excess of food intake over food requirement. The food intake may be excessive because of a reduction in metabolic needs effected through a depression of thyroid or of gonad activity or of both together, while hunger is maintained. It may be excessive in answer to an exaggerated hunger stimulated by a downward shifting blood glucose level

caused by the exaggerated consumption of carbohydrate in the tissues as in hypofunction of the hypophysis; or by effects resulting from exaggerated influences from the adrenal cortical hormone following an appropriate hypothalamic lesion. The changes in metabolism leading to obesity are quantitative rather than qualitative.

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HYPOPHYSIAL EOSINOPHIL CELL AND INSULIN SENSITIVITY

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Received for publication February 4, 1944

In a previous communication (Heinbecker, White and Rolf, 1942) it was shown that insulin sensitivity in dogs is to be attributed solely to a loss of the adenohypophysis. Herein is presented further evidence to show that such sensitivity is due to the loss of the eosinophil cells of the hypophysis only, and is unrelated to the loss of the basophil cells.

Studies were carried out on four groups of animals, normal dogs, simple and total hypophysectomy dogs and so-called puncture dogs. The total hypophysectomy dogs differed from the simple hypophysectomy dogs in that in the former the median eminence was removed as well as the anterior lobe, the pars tuberalis and the posterior lobe. To prepare the puncture dog the infundibular stem is severed through the oral approach, and then with a special instrument a puncture wound about 2.5 mm. in width is made in the midline in the posterior hypothalamus deep enough to sever the fibers running caudally from the paired paraventricular nucleus. The median eminence is also scooped out. Such dogs exhibit total diabetes insipidus and become quite obese. When they are sacrificed and the hypophysis serially sectioned and stained for cell types according to the method of Rasmussen, the eosinophil cells are normal and relatively increased in number due to the fact that the basophil cells are almost completely absent (Heinbecker, White and Rolf, 1944). No actual counts of the eosinophils have been made. The few basophils remaining have an agranular turbid cytoplasm.

Insulin sensitivity tests were carried out on six dogs of each of the groups according to a method previously used by Heinbecker, Somogyi and Weichselbaum (1937). Marked insulin sensitivity was present in the simple and total hypophysectomy dogs, but the puncture dogs responded similarly to the normal, and thus were not insulin sensitive (fig. 1). The functional capacity of the hypophysial eosinophil cells in the puncture dogs was tested for according to a method described by White, Heinbecker and Rolf (1943) by diodrast and inulin clearance studies and found to be normal.

Because of the normality of the blood sugar response to exogenous insulin in the puncture dogs with practically all basophil cells absent from the hypophysis, it follows that insulin sensitivity when present because of hypophysectomy, is due to the loss of the eosinophil cells.

The basis for the basophil cell loss in the puncture dogs has not been established with certainty. A first suspicion naturally was that it was due to the stalk section. By actual experiment it was established that stalk section in itself does not cause basophil cell loss. It was not possible experimentally to exclude the

[1] Recipient of a grant-in-aid of research from the Commonwealth Fund.

possibility that the total or nearly total loss of pitressin forming tissue might be responsible. However, it is known that in woman marked diabetes insipidus may exist without obesity and without interference with ovarian follicular function, as proven by her capacity to bear children. Certain other influences may be drawn from clinical and experimental data. It is known that in cases of Cushing's syndrome due to an adrenal tumor, hyalin degeneration of the basophil cells occurs with regressive changes in the thyroid and gonads similar to those which follow hypophysectomy in dogs (Heinbecker, 1943). Dogs with hypothalamic lesions resulting in degeneration of the paired paraventricular nucleus and with basophil cell loss are sensitized to exogenous cortical hormone as demonstrated by the degree of its influence on renal circulation and on certain renal

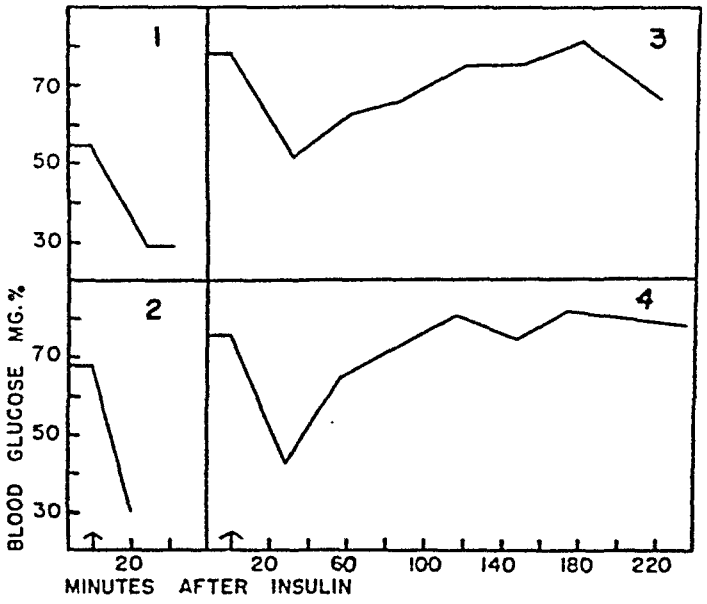

Fig. 1. Insulin response in dog 1, a total hypophysectomy dog; in dog 2, a simple hypophysectomy dog; in dog 3, a normal dog; and in dog 4, a puncture dog; 0.125 unit insulin per kilo given intravenously. In all animals without the adenohypophysis hypoglycemic manifestations developed necessitating discontinuance of the experiment and the administration of glucose.

tubular functions (Heinbecker, Rolf and White, 1943). Possibly therefore, an unbalanced or exaggerated influence from the adrenal cortex is responsible for the basophil depression in puncture dogs.

For this presentation the thesis is accepted that normally there are two secretory cell types in the anterior lobe of the hypophysis, eosinophils and basophils, and that the chromophobes represent a stage in the development of these two cell types. Autopsy studies of dogs of the puncture type in which the eosinophils are preserved have revealed that in them the adrenal glands do not atrophy. This is consistent with the interpretation that the insulin sensitivity following eosinophil cell loss in simple and total hypophysectomy dogs is related to the atrophy of the adrenal glands which develops. The progressive nature of such atrophy is illustrated in figure 2. The adrenals from a simple and total hypo-

physectomy dog approximately two and one-half years after operation show preservation of little other than the glomerular zone, whereas adrenal glands from such dogs at 8, 17 and 22 months after operation generally reveal a narrowing

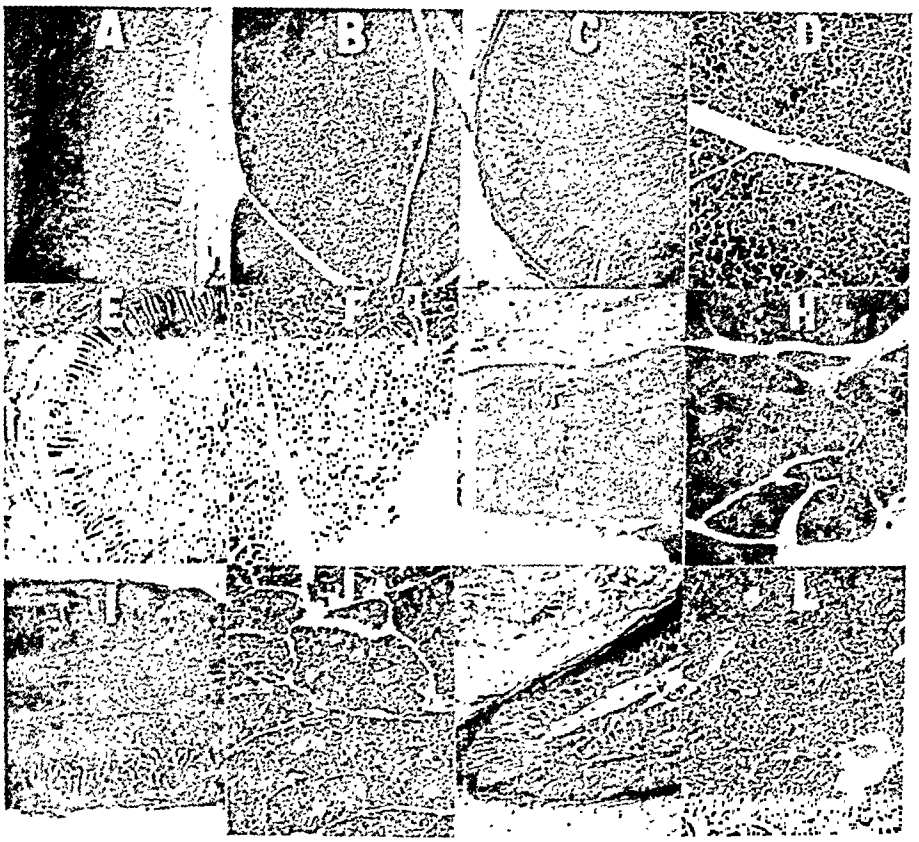

Fig. 2. A. Photomicrograph × 45 showing adrenal cortex from normal fat dog 3. B. Photomicrograph × 15 showing pancreas from dog 3. C. Photomicrograph × 15 showing adrenal cortex from puncture dog 4. Note normal adrenal cortex. D. Photomicrograph × 15 showing pancreas from dog 4. Note islets of Langerhans are few in number and small. E. Photomicrograph × 15 showing adrenal cortex from simple hypophysectomy dog 5, eight months after operation. Note moderate reduction in width of all layers. F. Photomicrograph × 15 showing pancreas from dog 5. Note normal islets. G. Photomicrograph × 15 showing adrenal cortex from simple hypophysectomy dog 2, 27 months after operation. Note the marked reduction of all the adrenal cortical layers except the glomerular zone which is wide but the cytoplasm of the cells is vacuolated and the nuclei stain lightly. H. Photomicrograph × 15 showing pancreas from dog 2. Islets are normal. I. Photomicrograph × 15 showing adrenal cortex from total hypophysectomy dog 1, 17 months after operation. Note considerable thinning of all the layers of the adrenal cortex. J. Photomicrograph × 15 showing pancreas from dog 1. Note hypertrophy and hyperplasia of the islets. K. Photomicrograph × 15 showing adrenal cortex from total hypophysectomy dog 6, 30 months after operation. Note the marked loss of all the layers except the glomerular zone. The cytoplasm is vacuolated and the nuclei are pale staining. L. Photomicrograph × 15 showing pancreas from 6. Islets are normal.

of the cortical layers with however preservation of all of them. It has previously been shown that the degree of insulin sensitivity present almost immediately after operation increases with time after hypophysectomy (Heinbecker, Somogyi

and Weichselbaum, 1937, loc. cit.) (fig. 3). Long and his co-workers (1940) have shown conclusively that the adrenal cortex is concerned in gluconeogenesis. Insulin sensitivity is considered to be primarily due to an inadequacy of gluconeogenesis. The depression of the adrenal cortex is regarded first as functional but later anatomical degeneration supervenes as well.

Study of the pancreases of various groups of dogs (fig. 3) shows that insulin sensitivity is not related to any consistent change in the islets of Langerhans. In insulin sensitive simple or total hypophysectomy dogs, the islets may be

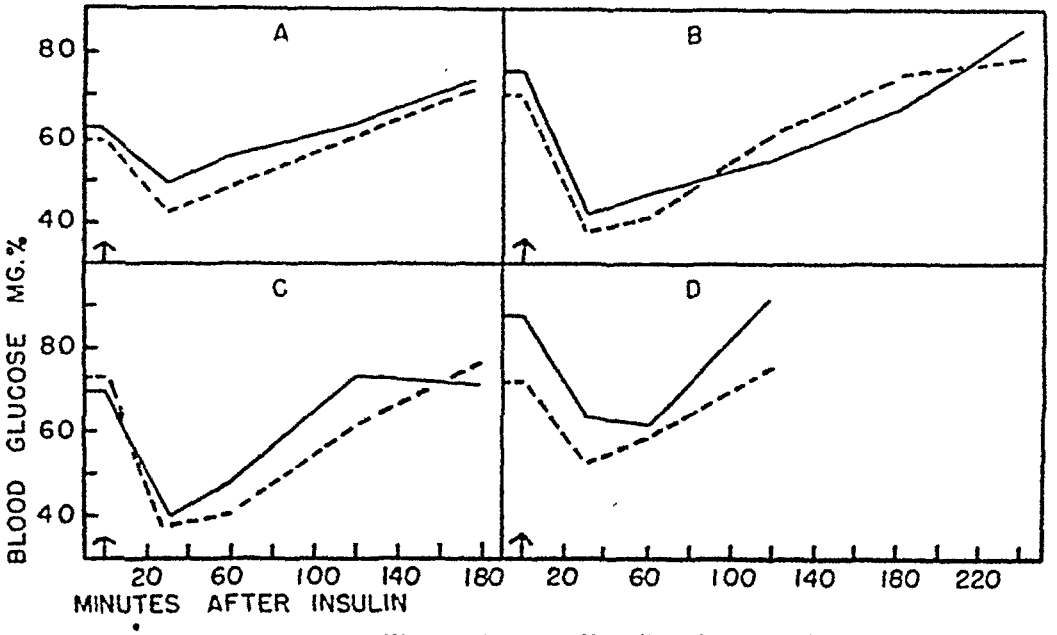

Fig. 3. A. Blood sugar curves illustrating insulin effect in a dog before and four weeks after hypophysectomy. Preoperative dosage was 0.5, postoperative 0.25 unit per kilo. The curves are approximately comparable. Normal curve unbroken curve, after hypophysectomy broken. B. Similar curves on a second dog four weeks after hypophysectomy. Insulin dosages the same as in A. C. Blood sugar curves from the same animal as for B. Dosages were 0.25 unit four weeks after operation and 0.125 unit per kilo one year after hypophysectomy. D. Blood sugar curves on same animal as for C. preoperatively and one year after operation. Preoperative dosage 0.25 unit, postoperative 0.06 unit per kilo. Note that both C. and D. indicate a postoperative insulin effect approximately four times that found for this dog preoperatively.

normal, atrophic or hypertrophic. Likewise, in normal and in puncture dogs which are not insulin sensitive, the islets may be normal, atrophic or hypertrophic.

Microscopically the thyroid gland and the gonads are similar in hypophysectomized and puncture dogs. In the thyroid there is a depression of colloid secretion, in the ovary the follicles do not mature and in the testis spermatogenesis does not proceed to completion. These changes are considered the result ofthe hypophysial basophil cell loss inasmuch as the eosinophil cells are well preserved in the puncture dog. Insulin sensitivity being present in the hypophysectomized dog and absent in the puncture dog it follows that it primarily is not effected through such regressive changes in the thyroid or in the gonads.

SUMMARY

Insulin sensitivity is attributed to a loss of the eosinophil cells of the anterior lobe of the hypophysis. Their loss results first in a functional depression of the adrenal cortex and later in progressive atrophy.

The progressive increase in insulin sensitivity with time after hypophysectomy is correlated with the progressive atrophy of the adrenal cortex.

Insulin sensitivity is not found to be associated with any consistent state of the islets of Langerhans. It is essentially unrelated to regressive changes in the thyroid and in the gonads.

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LOCAL LOSS OF FLUID AND PROTEIN IN EXPERIMENTAL SHOCK: RELATION TO DECREASE OF PLASMA VOLUME AND TOTAL CIRCULATING PROTEIN

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Received for publication March 20, 1944

The development of a body of evidence indicating that local loss of fluid is the cause of traumatic shock was apparently initiated by Blalock (1) in 1931. In dogs he showed that traumatized extremities contained fluid amounting to around 4 per cent of the body weight, and that a loss of fluid from the blood volume of this amount would be sufficient to produce shock. Investigating a form of experimental shock produced by subcutaneous injection of hypertonic saline, Davis (2) also found a marked local fluid lost, quite adequate in amount to produce shock. In these latter experiments the fluid lost was principally fluid of the plasma and was relatively low in protein content. Davis further showed that the plasma volume reduction, calculated by hematocrit changes, in dogs shocked by this method, was less than the amount of fluid recovered from the edematous leg. He therefore concluded that there was addition of fluid to the plasma during the collection of fluid in the leg. The determination of plasma volume changes in dogs by hematocrit changes involves errors of considerable magnitude, however, which unfortunately partly invalidates this important experiment. Katz, Shleser, Asher and Perlow (3) contribute significant evidence to the problem of the relationship of local fluid loss to the cause of shock. These workers showed that shock, which usually followed venous occlusion of the hind leg, was prevented by the application of a cast. The cast prevented the local fluid loss which otherwise would have occurred, and therefore, shock failing to appear, the conclusion was reached that local fluid loss was the sole factor in the development of this type of shock. This evidence would have been more acceptable if it had been shown that thrombosis of the veins had not taken place during the venous occlusion and period of cast application, since it might be argued that theoretical products of tissue damage were not liberated into the general circulation because of permanent venous obliteration. Swingle, Remington, Kleinberg, Drill and Eversole (4) however presented similar data in which shock after release of a tourniquet was prevented by bandaging the leg. In these experiments, an intact venous circulation was demonstrated to exist by the entrance of dye into the general circulation when it was injected into the veins of the leg. Duncan and Blalock (5) found that shock from crushing a leg was prevented by application of a pressure cuff, further indicating the significance of local fluid loss in the development of shock.

Another type of approach to the problem was utilized by Fine and Seligman (6, 7). Using radioactive tagged protein these workers found that all of the

protein disappearing from the circulating blood in experimental hemorrhage and shock was accounted for by that removed from the blood by hemorrhage, or that collecting in the damaged extremity.

In the present report further evidence is presented which indicates that local loss of fluid is the mechanism by which traumatic shock is produced. The aim of this study is to establish a quantitative correlation between the decrease of the plasma volume and total circulating protein, and the amount of fluid and protein which collect in the edematous leg of traumatic shock.

METHODS. Ten fasting normal dogs were used as the basis of this study. The animals were anesthetized with pentobarbital-sodium and a control plasma volume and total circulating protein were determined. Shock of varying grade was produced in each animal by a combination of application and subsequent release of a tight tourniquet to a hind leg, and blunt trauma. The tourniquet was left on for two to five hours. Trauma was applied to the medial aspect of the thigh with a blunt hammer after the tourniquet was released. The presence of shock was determined by blood pressure readings on a mercury manometer connected with a cannula in the carotid artery. The course of shock was followed by the same means. Shock of some variable degree was produced in each animal, as disclosed by a reduction of systolic blood pressure to 85 mm. Hg or less over a considerable period of time. In some animals shock was mild, and recovery, at least temporary, would have occurred; in others the shock was fatal. During the period of well developed shock a second plasma volume and total circulating protein were determined, after which the animal was sacrificed. The average duration of shock was 2.2 hours. The hind portion of the animal was then transected at about the level of the 3rd lumbar vertebra, and after removal of the viscera, the hind portion was carefully bisected. The excess weight of the traumatized leg over the normal was taken to represent the amount of local fluid loss. Fluid was then removed from two or more areas of the traumatized leg for determination of hemoglobin and protein content; the average of these figures was taken to represent the amount of hemoglobin and of protein in the fluid lost locally. The recovered fluid of different animals was found to vary in its hemoglobin content. In some instances this was almost negligible, while in others the fluid was grossly bloody. The amount of plasma fluid in the leg was determined by correction for red cell volume as represented by the amount of hemoglobin present.

Plasma volume was determined by the Evans blue dye method (8). Disappearance of the dye was plotted against the square root of time as advocated by King, Cole and Oppenheimer (9). Correction for hemoglobin in the plasma, which was invariably present, during shock, was made through the use of two monochromatic filters, viz., 620 and 540. Plasma protein was determined by the method of Greenberg (10) as adapted to the Lumetron photoelectric colorimeter.

RESULTS. The results are shown in table 1. The plasma volume decrease varied from 80 to 342 cc. and averaged 187 cc. which is 1.93 per cent of the average weight of these animals and 36.3 per cent of the original plasma volume.

In comparison to this there was a loss of from 183 to 387 cc. of fluid into the traumatized leg (exclusive of red cell volume), an average of 280 cc. or 2.89 per cent of the average weights and amounting to 54.1 per cent of the control plasma volume. Protein loss into the leg varied from 5.25 grams to 18.5 grams, averaging 12.6 grams which represented 34.2 per cent of the control protein. The amount of protein disappearing from the plasma varied from 6.7 to 17.8 grams, averaging 11.8 grams, slightly less than that lost into the leg. This decrease of total circulating protein represented 32 per cent of the original circulating protein.

TABLE 1

DOG NO.	WEIGHT	CONTROL PLASMA VOLUME	SHOCK PLASMA VOLUME	PLASMA VOLUME DECREASE	FLUID LOST IN LEG	CONTROL PROTEIN	SHOCK PROTEIN	PROTEIN LOST FROM BLOOD	PROTEIN LOST INTO LEG
	kgm.	*cc.*	*cc.*	*cc.*	*cc.*	*gm.*	*gm.*	*gm.*	*gm.*
1	11.1	627	363	264	367	37.3	24.4	12.9	14.9
2	11.0	467	338	129	185	32.7	24.6	8.1	6.7
3	6.6	370	267	103	118	24.4	17.7	6.7	5.3
4	13.3	704	362	342	387	49.5	28.4	21.1	18.5
5	8.1	412	332	80	183	26.4	22.4	4.0	6.9
6	10.9	627	485	142	285	46.0	31.6	14.4	12.8
7	8.4	415	255	160	321	23.9	15.3	8.6	16.0
8	9.2	540	308	232	280	34.3	19.8	14.5	13.2
9	9.2	495	218	277	363	30.7	12.9	17.8	17.1
10	9.0	515	370	145	320	33.0	23.2	9.8	14.4
Average	9.68	517	330	187	280	36.8	22.0	11.8	12.6

Data on plasma volume, total circulating protein, and local collections of fluid and protein.

Discussion. The results clearly indicate that there is a greater loss of fluid locally than occurs from the plasma volume itself. This is interpreted to mean that the significant disposition of the lost plasma volume is locally at the site of damage. It furthermore shows that, instead of a further loss of plasma occurring in other parts of the body, there actually is a certain degree of hemodilution in the phase of development of traumatic shock. That this process, however, is continuous throughout all phases of shock is not contended from these findings, and it is entirely possible, though not necessarily likely, that in the later stages of shock a loss of fluid other than locally may occur. This possibility does not decrease the certainty of the finding here that in the stage of development of shock, all of the fluid lost from the circulating blood collects at the site of trauma, and that this defect has been partially corrected by addition of fluid to the plasma from other fluid reservoirs. Except that smaller plasma volume losses were encountered in the milder instances of shock, the data show no significant variation in the instances of mild and severe shock. In essence, these findings are considered to be corroborative and additive to those of Blalock (1) and Davis (2) and they further extend the certainty of local fluid loss as the essential cause of traumatic shock. The results are also apparently contrary, to the theory of generalized increase of capillary permeability in traumatic shock.

There was found to be considerably less addition of protein to the blood-stream than fluid since 11.8 grams represented the average plasma protein decrease and 12.6 grams the average amount of protein lost locally. The difference is probably within the range of error and must be considered to indicate that, although fluid is added to the circulation from the interstitial reservoir, or the cells, or both, it is poor in protein content. This is quite in accord with results of others in regard to hemodilution after hemorrhage.

It will be noted that the amount of fluid lost in the leg is somewhat less than the figures of Blalock, Davis and others. This is due to the fact that our figures represent only plasma loss, correction having been made for red blood cell content. If the total weight difference were taken in these experiments it would be around 4 per cent of the body weight. These data are not presented since they are not related to the question under discussion.

SUMMARY AND CONCLUSIONS

In ten animals shocked by application and subsequent release of a tourniquet combined with blunt trauma it was found that the amount of fluid lost locally exceeded the plasma volume decrease and that the amount of protein lost locally slightly exceeded that lost from the blood stream. It is concluded that in the development of shock the plasma volume decrease is due entirely to local fluid loss and that fluid loss from capillaries all over the body is excluded as a possibility. Furthermore, hemodilution was found to take place. Since the total circulating protein was not appreciably augmented by this hemodilution, it is concluded that fluid entering the plasma is poor in protein content.

Aside from the negative evidence for demonstration of the toxic theory of traumatic shock, three separate but related lines of evidence may now be stated which seemingly irrevocably indicate that the mechanism of traumatic shock in dogs is local loss of fluid. These are: 1, loss of fluid into the damaged leg amounts to about 4 per cent of the body weight which is sufficient per se to lead to shock (Blalock and Davis); 2, prevention of local fluid loss after damaging the leg prevents the occurrence of shock (Katz and others, Swingle and others, and Duncan and Blalock); and 3, the measured fluid lost in the leg actually exceeds the measured decrease of plasma volume indicating that generalized capillary damage and fluid and protein loss do not occur in the phase of development of shock, but instead, fluid is added to the plasma as a compensatory phenomenon.

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FACTORS INFLUENCING CHLORIDE CONCENTRATION IN HUMAN SWEAT[1]

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Received for publication March 27, 1944

The factors which determine the concentration of chloride in human sweat are as yet incompletely understood even though they have been the subject of considerable research. This circumstance is largely attributable to the technical difficulties involved. The collection of a pure, representative sample of sweat and the simultaneous recording of pertinent data on the internal and external environment of the subject require many precautions which frequently have not been observed. Important factors which obviously might affect the function of the sweat glands, particularly body and skin temperatures, have not been studied systematically or have been treated only qualitatively.

A summary of all the pertinent literature in this field is presented in table 1. Only on four points (identified in the table by asterisks) is there complete agreement by all workers. These are that the concentration of chloride in sweat: (1) tends to increase as work is prolonged, (2) varies markedly between individuals, (3) varies in different regions of the body, and (4) varies inversely as the supply of drinking water. On two points (identified by daggers) more observers affirm than deny that the concentration of chloride in sweat: (1) decreases during the course of acclimatization, and (2) increases as the rate of sweating increases. On three other points (identified by double daggers) observers are almost equally divided in their conclusions. These are that the concentration of chloride in sweat: (1) varies directly as the body temperature, (2) varies with the intake of salt in the diet and (3) varies with the plasma chloride. With respect to the important factor of skin temperature observations have been scanty and qualitative in nature. The two observers who have worked on this point agree that local warming of an arm increases the chloride concentration in its sweat. A critical consideration of the above points will be presented in the discussion of this paper, where it will be pointed out that some of the published work is open to an interpretation other than that commonly given.

The present authors were led to a reinvestigation of the problem by a number of considerations. First, it would be desirable from the standpoint of practical nutrition to be able to predict chloride requirements under various physiological and environmental conditions. Second, there is the possibility that individual differences in the concentration of chloride in sweat might be correlated with fitness for working in hot environments. Finally, clarification of the factors controlling the activity of the sweat glands is a matter of considerable theoretical interest in general physiology.

[1] This work was done under a contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development and the President and Fellows of Harvard College.

TABLE 1

Survey of literature on factors affecting sweat chloride

OBSERVATION ON CHLORIDE CONCENTRATION IN SWEAT	AUTHORS
Individual and regional differences	
*Varies markedly between individuals	Koriakina, Kossowskaja and Krestownikoff (1930); Whitehouse (1931. 1935); Chatron (1933); Dill, Jones, Edwards and Oberg (1933); Dill, Hall and Edwards (1938)
*Varies in samples from different parts of the body	Kittsteiner (1911); Adolph (1923); Mickelsen and Keys (1943)
Rate and duration of sweating	
†Increases as the rate of sweating increases	Kittsteiner (1911, 1913); Dill, Hall and Edwards (1938)
Tends to fall with increased sweat production	Lee, Murray, Simmonds and Atherton (1941)
*Increases as duration of experiment is prolonged	Viale (1913); Adolph (1923); Hancock, Whitehouse and Haldane (1929); Marschak and Klaus (1929); Whitehouse (1931); Hardt and Palmer (1937); Talbert, Harris, Finkle and Silvers (1926)
Acclimatization	
†Decreases during the course of acclimatization	Dill, Jones, Edwards and Oberg (1933); Talbott, Edwards, Dill and Drastich (1933); Daly and Dill (1937); Mezincescu (1937); Dill, Hall and Edwards (1938); Mickelsen and Keys (1943)
No effect of acclimatization	McCance (1938); Lee, Murray, Simmonds and Atherton (1941)
Body and skin temperature	
‡Varies directly as the rectal and skin temperature	Lehmann and Szakáll (1939)
No relation to rectal temperature	Taylor, Henschel, Mickelsen and Keys (1943)
Obtained a concentrated sweat by local warming of the arm or a dilute by local cooling	Cramer (1890); Kittsteiner (1911, 1913)

TABLE 1—*Concluded*

OBSERVATION ON CHLORIDE CONCENTRATION IN SWEAT	AUTHORS
Diet and water intake	
‡Decreased by intake of salt	Molchanova, Legun, Redina, Chechel'nitzkaya and Frolova (1936); Lehmann and Szakáll (1939)
‡Varies directly as salt intake	Kittsteiner (1911, 1913); Hancock, Whitehouse and Haldane (1929); McCance (1938)
‡Practically independent of diet	Berry (1915); Cuthbertson and Guthrie (1934)
Reduced by substituting saline solution for drinking water	Lee, Murray, Simmonds and Atherton (1941)
*Varies inversely as the supply of drinking water	Viale (1913); Hancock, Whitehouse and Haldane (1929); Lee, Murray, Simmonds and Atherton (1941)
Blood chloride	
‡Some correlation between blood and sweat chloride	Talbert and Haugen (1927)
Independent of moderate variations in plasma chloride	Mickelsen and Keys (1943)
Other factors	
Higher in hot dry than in hot wet environments	Lee, Murray, Simmonds and Atherton (1943)
Increased by an increase in work output at constant temperature and humidity or by an increased temperature with constant work output	Moss (1923)

METHODS. The present report is based on two types of experiment which will be considered separately in this section.

1. *Outdoor experiments.* In the summer of 1942 a group of sixteen healthy young men made a series of marches covering 10 to 20 miles per day over a flat course outdoors at fixed paces which were on different occasions 3, 4, 4½ and 5 m.p.h. Standard summer suntan uniforms were worn and the subjects lived on their usual normal diet. Five subjects were studied intensively and repeatedly throughout the course of their acclimatization. A rigid routine was followed in collecting sweat in order to avoid evaporation, dilution and contamination. Every four miles an arm was washed in clean water, dried with a clean towel and covered with an obstetrical rubber glove which was closed at the elbow with rubber bands fitting snugly but not tightly enough to impede circulation. The

following measurements were made periodically in each experiment: (1) environmental temperature and relative humidity with a sling psychrometer, (2) pulse rate by palpation, (3) rectal temperature with standard clinical thermometers, (4) rate of sweating by gross loss in nude weight and (5) sweat chloride by the method of Volhard-Harvey (1878, 1910).

2. *Experiments in the heated room.* In this series of observations six healthy young men marched three times a week on a motor driven treadmill at 3.5 m.p.h. up grades which will be specified in the tables. Depending upon the experimental conditions of temperature and humidity which were constant in individual experiments, the nude subjects marched anywhere from one to five hours with a ten minute rest in each hour. In one series dry bulb temperatures were 100°, 110° and 120°F. with a relative humidity of about 30 per cent while in another they were 80°, 85°, 90° and 95°F. and 85 per cent humidity respectively. Pulse rate, rectal temperature, rate of sweating and sweat chloride were determined as in the outdoor experiments and the same precautions were observed in collecting sweat. A further refinement in technique consisted in fitting the little fingers of the gloves with glass spigots.

Additional observations were made as follows: *a.* Skin temperature was measured by means of copper-constantan thermocouples and a galvanometer. It was proved that when both arms are in rubber gloves, the temperature of corresponding regions of skin is almost always the same on both arms. Therefore, in order to avoid error from evaporation and contamination, samples of sweat were always collected from the left arm and skin temperature was measured on the lateral aspect of the right wrist. *b.* Samples of venous blood were drawn periodically from an antecubital vein. In some series of experiments plasma was prepared from heparinized whole blood and in others, serum from clotted blood. Total protein was estimated by the method of Ma and Zuazaga (1942) and chloride by the method of Volhard-Harvey (1878, 1910).

Results and discussion. The treatment of material in this section will conform to the following plan: First, individual experiments (tables 2 to 6) will be treated in relation to previous work (table 1). Second, we shall justify the viewpoint that, disregarding personal idiosyncrasies and local variations, a combination of three physiological measurements, i.e., body temperature, skin temperature and rate of sweating, satisfactorily accounts for the summated effects of all the variables previous workers have correlated with the level of sweat chloride. Finally, the hypothesis will be presented that the level of chloride in sweat is regulated by the interplay of a central factor, a peripheral factor and individual idiosyncrasy.

The results of the outdoor and indoor experiments will be discussed separately except in the cases of acclimatization and physical fitness.

1. *Outdoor experiments.* In 25 experiments on the 16 subjects, 97 samples of sweat were collected. Variations in the weather gave wide ranges of rectal temperatures and rates of sweating. Table 2 summarizes two experiments on 11 subjects who marched rapidly under relatively mild conditions half receiving 9 grams of salt before each experiment. This was the calculated loss of salt for

the amount of sweat anticipated. Four chief results were obtained. First, with three exceptions higher chloride concentrations accompanied higher rectal temperatures and rates of sweating. Second, there were characteristic differences in chloride concentration of the sweat from one individual to the next even when their rectal temperatures were the same. There is agreement among all workers concerning individual variations and the matter will not be discussed although it is apparent in figure 1 and tables 3, 4, 5 and 7. Third, with the

TABLE 2

Sweat chloride, rectal temperature and rate of sweating in marching outdoors

(First experiment, 10 miles at 4½ m.p.h., dry bulb 86°F., wet bulb 73°F. Second experiment, 10 miles at 4½ m.p.h., dry bulb 77°F., wet bulb 64°F.)

SUBJECT	SWEAT CHLORIDE		RECTAL TEMPERATURE AT END		RATE OF SWEATING	
	Expt. 1* No salt	Expt. 2* Salt	Expt. 1 No salt	Expt. 2 Salt	Expt. 1 No salt	Expt. 2 Salt
	meq./l.		*°F.*		*Total liters*	
Group I						
S. R.	23	20	100.9	100.8	2.96	2.15
G. T.	23	25	101.0	100.4	2.96	2.37
R. J.	53	48	101.4	100.7	2.66	2.41
C. G.	20	18	101.8	101.2	2.80	2.05
J. C.	33	29	101.8	101.4	2.70	2.74
J. B.	47	37	102.0	101.2	2.65	1.75
Average	33	30	101.5	101.0	2.79	2.25
	Salt	No salt	Salt	No salt	Salt	No salt
Group II						
T. D.	32	27	101.0	100.1	2.52	2.47
H. B.	51	31	101.6	100.8	2.73	2.30
A. G.	40	35	101.8	101.2	3.16	2.29
S. P.	53	48	101.8	100.6	3.09	2.02
F. C.	53	57	102.4	101.5	3.84	3.05
Average	46	40	101.7	100.8	3.07	2.43

* In both experiments all subjects drank water equal to approximately 80 per cent of their sweat loss. In experiment 1, group II received 9 grams of NaCl in enteric coated capsules one to two hours before starting to march. In experiment 2, group I received salt in the same dosage.

terminal rectal temperature as an index of physical fitness for this type of work there was no correlation between sweat chloride and physical fitness. Finally, administration of salt in large single doses was without significant effect on the sweat chloride concentration.

Typical results relating sweat chloride and rectal temperature for two of the most intensively studied subjects are plotted in figure 1. Data for eleven experiments are plotted regardless of experimental conditions or degree of acclima-

tization. Inspection reveals the close correlation between rectal temperature and sweat chloride. The same correlation held with rate of sweating.

Further evidence on the relation between sweat chloride and physical fitness was obtained in the hot room. Table 3 gives typical data for five fully acclimatized subjects performing the same fixed task in moist heat. As judged by terminal rectal temperature and pulse rate their fitness bore no correlation to the concentration of chloride in their sweat.

TABLE 3

Sweat chloride and physical fitness of fully acclimatized subjects for marching in humid heat
(All subjects marched 70 minutes without water at 95°F., 83 per cent relative humidity, grade 2.5 per cent, speed 3.5 m.p.h., wind 5 m.p.h.)

SUBJECT	SWEAT CHLORIDE	RECTAL TEMPERATURE AT END	PULSE RATE AT END
	meq./l.	°F.	*beats/min.*
G. P.	74	101.6	128
J. S.	66	101.9	142
J. P.	86	102.5	150
F. S.	103	103.0	170
M. C.	65	103.2	166

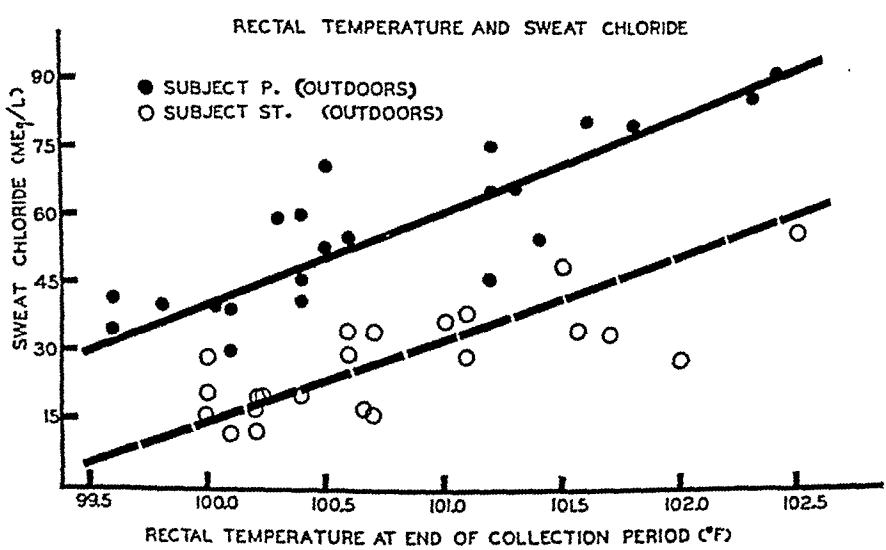

Fig. 1

2. *Experiments in the heated room.* Table 4 summarizes pertinent data from two subjects on sweat chloride as affected by the ingestion of water during work in moist heat. These fully acclimatized men were living on a constant diet containing in the case of J. S. 25 grams of chloride (as NaCl) and in the case of R. W. 19 grams of chloride (as NaCl). They marched 3 hours each day in the hot room under the conditions indicated in the table. Every second day they marched without water to drink and on the alternate days they drank every 15 minutes water equal in amount to two-thirds the loss of sweat. Experience has

shown that this rate of drinking is the maximal well tolerated by most men. Five points are clear from the table. First, sweat chloride increased as work was prolonged. Second, serum chloride tended to increase when water was withheld and tended to decrease when it was drunk. Third, there was no apparent correlaton between serum and sweat chlorides. Fourth, as work was prolonged, there was an increase in serum protein, skin temperature and rectal temperature and a decrease in the rate of sweating. Finally, administration of water enabled the subjects to maintain a low rectal temperature and at the same time tended to prevent the rise in serum protein and skin temperature and the fall in rate of sweating. (The beneficial effects of water during work in the heat are discussed by Johnson, 1943.)

TABLE 4

The effects of ingesting water on sweat chloride, composition of blood and performance during marching in moist heat

(Dry bulb 90°F.; relative humidity 83 per cent; pace 3.5 m.p.h.; grade 4 per cent; 10 min. rest in each hour; subjects on a constant diet throughout; each figure the average of three experiments)

EXPERIMENTAL CONDITIONS	TIME	SWEAT CHLORIDE	SERUM		SKIN TEMP.	RECTAL TEMP.	RATE OF SWEATING
			Chloride	Protein			
Subject J. S.							
	hrs. marched	*meq./l.*	*meq./l.*	*gms./100 cc.*	*°F.*	*°F.*	*l./hr.*
No water	1	56	107	6.5	98.3	100.7	1.40
	3	70	108	7.8	98.9	102.2	1.25
Water	1	51	104	6.3	97.4	100.4	1.34
	3	60	102	7.0	97.8	100.7	1.24
Subject R. W.							
No water	1	39	107	6.2	98.9	101.3	1.57
	3	46	108	7.6	99.9	103.2	1.25
Water	1	36	104	6.2	98.2	100.8	1.49
	3	38	103	7.2	98.7	101.3	1.46

The effects of drinking salted water are illustrated in table 6 for three fully acclimatized subjects marching under hot, dry conditions. Fluid was drunk every fifteen minutes at a rate equal to sweat loss. In one set of experiments plain tap water was used, in another 0.2 per cent saline. Consequently, in the latter set of experiments the subjects consumed approximately 6 grams of sodium chloride during the four hours of marching. The three main conclusions to be drawn are: First, that at equivalent rectal temperatures, skin temperatures, rates of sweating and times of marching the sweat chloride was lowered by saline; second, that the increase in sweat chloride typical of experiments in which water alone was drunk was prevented to some extent by saline of equal volume; and third, saline in comparison with an equal volume of water lessened the total

rate of sweating at the beginning of work and tended to prevent the decrease in rate of sweating characteristic of prolonged work. Lee and colleagues (1941) have previously reached similar conclusions concerning the sweat chloride but not the rate of sweating. It will be recalled that in our own outdoor experiments salt had no effect on sweat chloride. It should be pointed out first, that in the outdoor experiments salt tablets were administered before the experiment started but in the indoor experiments saline was drunk every fifteen minutes. Second, the values for sweat chloride in the outdoor experiments were very low in comparison to those in the hot room, a phenomenon which has been noted repeatedly by us and by other workers. It is possible that in our outdoor experiments the chloride secreted by the sweat glands was already near its minimal concentration and could not be expected to fall much lower.

The effect of acclimatization on sweat chloride is illustrated in table 6. Five young men living on their usual normal diet were subjected to a course of acclimatization as described by Robinson and colleagues (1943). G. P., J. P., and J. S. were exposed to dry heat, M. C. and F. S. to moist heat. Typical data are shown in table 6. It is apparent that under equivalent physiological conditions of rectal temperature and skin temperature, the chloride content of the sweat is essentially the same regardless of the degree of acclimatization. Complementary, but less complete, data are shown in the same table for three subjects marching outdoors. In these cases the chloride concentration in the sweat is approximately the same before and after acclimatization at the same rectal temperature, and an increased rectal temperature is associated with an increased sweat chloride.

It has been repeatedly stated that the concentration of chloride in sweat decreases with acclimatization (table 1). We can now suggest a reasonable interpretation for this phenomenon on the basis of changes in rectal and skin temperatures. It has been established that striking and rapid improvement in performance of a fixed task is found in the course of acclimatization. There is a characteristic day to day decrease in the rectal and skin temperatures during the performance of such a fixed task (Robinson, Belding, Turrell and Horvath, 1943). According to the interpretation now presented, the sweat chloride at a given skin and rectal temperature is relatively constant. The subject undergoing acclimatization theoretically ought to produce a progressively more dilute sweat during the daily performance of a fixed task, since his skin and rectal temperatures are lower. The present work shows experimentally that if at the end of the period of acclimatization, he does chance to attain a high rectal and skin temperature, his sweat chloride once more reaches its initial high levels. The practical implications of acclimatization remain unaffected by the above interpretation. There is doubtless a net daily saving in chloride after acclimatization, unless the subject pushes himself daily to high rectal and skin temperatures.

Plasma chloride and plasma protein may be dismissed briefly. Tables 4, 5 and 6 demonstrate the independence of sweat and plasma chlorides. Although plasma protein and sweat chloride both tend to rise steadily during a prolonged experiment, as shown in table 4, and in a large mass of other data

not presented here, comparison of the two for equivalent rectal temperatures, skin temperatures and rates of sweating revealed no significant correlation.

3. *Evaluation of factors correlated with sweat chloride.* Reference to table 1 shows that for men in a normal state of nutrition the present work confirms and extends the conclusions that the concentration of chloride in sweat: (a) increases as work is prolonged, (b) varies markedly between individuals, (c) varies inversely as the supply of drinking water, (d) decreases during the course of acclimatization, but only as the rectal and skin temperatures decrease, (e) increases as the rate of sweating increases, (f) varies directly as the body temperature, (g) varies inversely with the intake of salt and (h) is not correlated with the plasma chloride. In addition we have found no correlation between sweat chloride and (i) plasma protein or (j) physical fitness but a close correlation with (k) skin temperature. In order to facilitate further generalizations we suggest that the above eleven factors fall into three categories: first, factors which within wide limits are without demonstrable effect; second, those which

TABLE 5

Sweat chloride as affected by drinking solutions of sodium chloride during marching
(The subjects marched 13 miles at 100°F., relative humidity 35 per cent, grade 2.5 per cent, speed 3.5 m.p.h., wind 5 m.p.h. with 10 minutes rest in each hour. Water or saline was drunk as described in text. Each figure the average for 3 subjects.)

EXPERIMENTAL CONDITIONS	PERIOD OF MARCH	SWEAT CHLORIDE	RECTAL TEMP.	SKIN TEMP.	RATE OF SWEATING	SERUM CHLORIDE
	hrs.	*meq./l.*	*°F.*	*°F.*	*l./hr.*	*meq./l*
Water	1	83	100.8 ± 0.6	99.1 ± 1.0	0.95	102
	4	96			0.80	98
Saline	1	72	101.1 ± 0.8	99.1 ± 0.9	0.90	101
	4	79			0.80	99

affect sweat chloride directly; and third, those which affect sweat chloride by their influence on the body as a whole. In the case of subjects subsisting on a diet adequate in all respects we place in the first category plasma chloride, plasma protein and physical fitness because of their demonstrated lack of correlation with sweat chloride. In the second category we place body temperature, skin temperature, rate of sweating, individual variations and regional variations because in our experience they are invariably correlated with sweat chloride when they vary independently of all other factors. Finally, it is justifiable to consider as indirect factors duration of work, intake of water, intake of salt and acclimatization for the following reasons: These four factors are all obviously related to heat balance. As shown above (tables 4 and 5) prolongation of work in the heat, especially in the absence of water, is usually associated with steadily rising rectal and skin temperatures and a decreasing rate of sweating. Administration of water during work permits the maintenance of relatively low rectal and skin temperatures associated with a sustained rate of sweating (table 4). Administra-

tion of saline during work apparently decreases the rate of sweating at a given rectal temperature (table 5). Acclimatization (Robinson and colleagues, 1943) is characterized by lower rectal and skin temperatures in the performance of a fixed task but apparently has no effect on sweat chloride provided observations are made at equivalent skin and rectal temperatures. The above considerations lead to the conclusion that from the physiological standpoint observations on body temperature, skin temperature and rate of sweating account for the summated effects of all the physiologically dependent mechanisms affecting sweat chloride with the exceptions of individual and regional variations.

Under most circumstances skin temperature, rectal temperature and rate of sweating do not vary independently but in general vary together. In the present series of observations two general methods have been employed in the attempt to dissociate these factors. First, on different days and sometimes in the course of the same day alterations were made in one or more of the following: environmental temperature, severity of work, degree of hydration, and duration of work. In such a series of experiments, skin temperature, rectal temperature and rate of sweating all show wide variations, and by selecting periods in which two of the three were by chance the same, correlation of sweat chloride could be made with the third variant. In the second type of experiment specific attempts were made to alter one variable without changing the other two. Skin temperature was altered by immersion of the gloved arm in a water bath at any desired temperature. Rate of sweating was controlled to some extent by changing environmental temperature or severity of work or both. Obviously, a high rectal temperature was always accompanied by a high rate of sweating but a low rectal temperature could sometimes be attained with a relatively high rate of sweating.

Representative data obtained by the above two methods are presented in table 7 which consists of three separate sections. In each section two of the three variables are constant while the third varies independently. The independent variables are skin temperature, rectal temperature and rate of sweating respectively in sections A, B and C. In examining the data it must be borne in mind that day-to-day variability introduces some uncontrolled irregularity. Comparison is made between paired values which satisfy the following criteria: the experiments compared were not more than one month apart; observations were at comparable times during the course of the experiments; and constancy was defined as a difference in rectal and skin temperatures of not more than 0.4°F. and a difference in rate of sweating of not more than 10 per cent. The three variables will be discussed separately, and then a general inference relating the three will be presented.

Section A demonstrates for two subjects that at a fixed rectal temperature and rate of sweating an increase in the local skin temperature is accompanied by an increase in sweat chloride. In 27 comparisons for six subjects 74 per cent showed this relation between skin temperature and sweat chloride.

Section B is probably inconclusive for two reasons. First, the amount of comparable data is undesirably small. Second, the usual relationship between

rectal temperature and rate of sweating is so close that it proved difficult to obtain paired values in which the rectal temperature alone varied significantly.

TABLE 6

Sweat chloride before and after acclimatization

SUBJECT	SWEAT CHLORIDE	SKIN TEMPERATURE	RECTAL TEMPERATURE	RATE OF SWEATING	SERUM CHLORIDE
Indoor experiments					
	meq./l.	*°F.*	*°F.*	*l./hr.*	*meq./l.*
G. P.					
Before*	97	98.1			
After*	86	94.0	100.2 ± 0.3	0.78 ± 0.03	103 ± 4
After*	114	98.0			
J. P.					
Before	112	99.2			
After	88	93.6	100.9 ± 0.2	1.02 ± 0.17	101 ± 1
After	109	99.1			
J. S.					
Before	65	98.6			
After	55	94.0	100.7 ± 0.4	1.01 ± 0.01	102 ± 1
After	60	98.5			
M. C.					
Before	82	101.3			
After	68	96.8	102.7 ± 0.7	1.34 ± 0.25	103 ± 1
After	85	99.5			
F. S.					
Before	72	100.9			
After	63	98.0	102.8 ± 0.7	1.24 ± 0.12	101 ± 1
After	74	100.0			
Outdoor experiments					
R. J.					
Before	18		100.5		
Before	43		102.2		
After	17		100.4		
After	43		102.2		
J. P.					
Before	45		101.0		
Before	83		102.0		
After	56		100.8		
After	77		102.0		

* Before and after in each case means before and after acclimatization.

Inspection of sections A, B and C shows that whereas it was possible to obtain wide independent variations of skin temperature and rate of sweating, the largest

TABLE 7

Sweat chloride as related independently to skin temperature, rectal temperature and rate of sweating

(Each horizontal line contains data from a single experiment. The lines grouped for comparison are from experiments in which two of the three variables were approximately constant.)

A — SKIN T. VARYING, RECTAL T. AND RATE OF SWEATING CONSTANT

Subject	Sweat chloride	Skin temp.	Rectal temp.	Rate of sweating
	meq./l.	*°F.*	*°F.*	*l./hr.*
F. S.	37	88.2	100.6±0.2	0.51±0.02
	38	90.3		
	42	94.7		
F. S.	37	88.2	100.9±0.1	0.55±0.02
	38	89.5		
	46	91.8		
	55	94.9		
	64	106.2		
F. S.	46	91.8	101.1±0.1	0.52±0.01
	69	94.3		
F. S.	98	93.0	101.2±0.1	0.50±0.01
	51	95.4		
F. S.	52	92.5	101.2±0.0	0.50±0.02
	69	94.3		
F. S.	63	98.0	102.1±0.1	1.32±0.02
	73	102.8		
F. S.	71	99.5	103.3±0.2	0.95±0.01
	76	102.4		
M. C.	30	86.5	100.5±0.1	0.59±0.01
	38	105.8		
M. C.	24	86.2	100.8±0.1	0.55±0.0
	32	89.0		
M. C.	49	80.5	100.9±0.2	0.96±0.02
	56	93.0		
M. C.	74	99.9	103.3±0.1	1.39±0.04
	82	101.3		
M. C.	60	94.9	103.4±0.0	1.47±0.03
	71	101.2		

B — RECTAL T. VARYING, SKIN T. AND RATE OF SWEATING CONSTANT

Subject	Sweat chloride	Skin temp.	Rectal temp.	Rate of sweating
	meq./l.	*°F.*	*°F.*	*l./hr.*
M. C.	65	87.4±0.0	101.0	0.47±0.01
	59		101.4	
M. C.	74	99.9±0.1	103.1	1.37±0.02
	69		103.5	
M. C.	77	100.1±0.1	102.1	1.73±0.02
	67		103.0	
M. C.	62	100.2±0.2	102.2	1.82±0.01
	65		103.2	
F. S.	73	88.3±0.1	100.4	0.47±0.01
	50		101.5	
F. S.	42	94.5±0.2	100.4	0.50±0.01
	69		101.2	

C — RATE OF SWEATING VARYING, SKIN T. AND RECTAL T. CONSTANT

F. S.	51	95.0±0.1	101.0±0.1	0.39
	55			0.55
F. S.	51	95.3±0.1	101.2±0.1	0.39
	51			0.50
F. S.	50	88.2±0.1	101.5±0.0	0.4[illegible]
	59			0.6[illegible]
F. S.	54	97.1±0.1	100.5±0.1	0.97
	56			1.07
F. S.	74	99.4±0.1	102.4±0.1	1.46
	76			1.70
F. S.	61	99.7±0.2	102.9±0.1	1.25
	71			1.40
F. S.	85	100.0±0.1	103.2±0.1	1.04
	57			1.18
	74			1.30
J. S.	43	97.1±0.2	100.3±0.1	1.29
	60			1.43
J. S.	42	97.0±0.2	100.6±0.2	1.24
	60			1.43
J. S.	56	98.7±0.2	100.6±0.1	1.01
	60			1.39
	67			1.57
J. S.	47	98.9±0.1	100.8±0.0	0.98
	53			1.09

independent variation in rectal temperature was 1.1°F. The data as they stand indicate that at a fixed skin temperature and rate of sweating a small change in rectal temperature is not accompanied by any consistent change in sweat chloride. It will be recalled that the outdoor data (fig. 1 and table 2) showed a close correlation between sweat chloride and both rectal temperature and rate of sweating. In the present hot room data the same relationship holds if we adopt the criteria used in preparing figure 1, namely, ignoring skin temperature and rate of sweating thereby obtaining a wide range of rectal temperatures. This is best seen in section A of figure 7 for subjects MC and FS by examining the values for sweat chloride as their rectal temperatures rose from 100.5° to 103.4°F.

Section C demonstrates for two subjects that at a constant skin and rectal temperature an increase in the rate of sweating is accompanied by an increase in the sweat chloride. In 17 comparisons for six subjects 77 per cent show this relationship. The data in section C are much more satisfactory than those in section B because it is fairly easy to obtain wide variations in the rate of sweating at a constant rectal temperature, but the reverse is not true.

4. *Hypothesis concerning regulation of chloride in sweat.* The correlations discussed above suggest that three primary mechanisms regulate the concentration of chloride in sweat. These are: (a) the peripheral factor of skin temperature; (b) a central factor of which rectal temperature and rate of sweating are probably the most important indices; and (c) the factor of individual idiosyncrasy. The general level of sweat chloride appears to be set by individual idiosyncrasy and by the central factor, increasing with increased rectal temperature and rate of sweating. Superimposed on this general level are fluctuations which appear to be correlated with the local skin temperature. The interplay of these mechanisms plausibly explains changes heretofore ascribed to duration of work, environmental conditions, intake of water, intake of salt and acclimatization.

SUMMARY

1. A study has been made of the concentration of chloride in human sweat in relation to factors previously reported to have a controlling influence. A survey of the literature revealed: complete agreement that sweat chloride (a) increases as work is prolonged, (b) varies between individuals, (c) varies in different regions of the body and (d) varies inversely as the supply of drinking water; majority agreement that sweat chloride (e) increases as the rate of sweating increases and (f) decreases during acclimatization; complete lack of agreement that sweat chloride is affected (g) by body temperature, (h) by intake of salt and (i) by plasma chloride; and insufficient or no attention to (j) skin temperature, (k) plasma protein and (l) the relation of sweat chloride to physical fitness for work in the heat.

2. Experiments were conducted on men marching out of doors in the summer time and in a heated room in the winter.

3. The present experiments confirm and extend conclusions (a), (b), (d),

and (e) in 1. above. In addition they indicate that the sweat chloride increases with increasing body temperatures, increases with local skin temperature, decreases more after ingestion of saline solution than after an equal volume of water and within wide limits is independent of plasma protein, plasma chloride and physical fitness.

4. It is suggested that three primary factors are concerned with the concentration of chloride in sweat. These are: (a) the local factor of skin temperature; (b) a central factor of which rectal temperature and rate of sweating are probably the most important indices; and (c) the factor of individual idiosyncrasies. The general level of sweat chloride appears to be dominated by this central factor, and increases with increased rectal temperature and rate of sweating. Superimposed on this general level are fluctuations which appear to be correlated with the local skin temperature. The interplay of these factors plausibly explains changes heretofore ascribed to duration of work, environmental conditions, intake of water, intake of salt and acclimatization.

5. A new interpretation is presented concerning sweat chloride during acclimatization. On the basis of 4 above one would theoretically expect a progressive lowering of the sweat chloride to be associated with the progressive decrease of rectal and skin temperatures which is characteristic of acclimatization in men performing a fixed daily task. Experimentally it was shown that if the rectal and skin temperatures were the same, the sweat chloride was the same before and after acclimatization.

Acknowledgments. We are greatly indebted to the 1st Service Command and to the Quartermaster Corps, U. S. Army for the services of Cpl. M. Castiglione, Cpl. J. Poulin, Pfc. A. Razoyk and Pfc. J. Stachelek who served as subjects and technical assistants in these experiments.

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THE EFFECT OF SODIUM THIOCYANATE ON INTESTINAL SECRETION IN THE DOG[1]

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Received for publication April 7, 1944

The recent interest in the possible function of carbonic anhydrase in the secretion of gastric juice (1) and of pancreatic juice (2) suggested experiments to determine the effect of thiocyanate on intestinal secretion. A preliminary report was made in 1941 (3). Thiocyanate inhibits the carbonic anhydrase catalysis of the hydration of carbon dioxide in vitro (1, 4). Davenport (1) found that feeding sodium thiocyanate to dogs with Pavlov pouches resulted in a low rate of secretion as well as a decrease in concentration of free acid, total acid and chloride. His explanation was that thiocyanate inhibited the carbonic anhydrase which is probably a part of the secretory mechanism for H^+ and Cl^-. More recently, Davenport stated that the concentration of thiocyanate in the blood and gastric secretion of the dogs with Pavlov pouches was insufficient to inhibit the carbonic anhydrase of the gastric mucosa, and there must be some other explanation for the inhibitory effect of the thiocyanate on gastric secretion (5).

Although the carbon dioxide content of the intestinal juice was unaffected by thiocyanate, and the theory of carbonic anhydrase inhibition by thiocyanate appeared to be inadequate for an explanation of Davenport's results on gastric secretion, an effect on the rate of intestinal secretion of fluid and enzymes as a result of thiocyanate administration was found which seemed worthy of further study.

METHODS. Dogs 1 and 2 had adjacent segments of the ileum taken about 10 cm. above the ileocecal valve transplanted to the two submammary surfaces of the skin. After about six months, during which time a good collateral circulation was established, the mesenteric pedicle was doubly ligated and cut in a second operation, so that the transplants thereafter had no direct nervous or vascular connection with the mesentery. It was in this second stage that these two animals were studied. This operative technique was described in an earlier paper (6). Dogs 3 and 4 each bore a single transplant of the colon. These colonic transplants were in the first stage, the original mesenteric blood vessels and nerves having been left intact. Dog 5 had a single fistula of the duodenum. Dogs 7 and 8 had Thiry-Vella fistulae of the jejunum.

The animals were set up in stalls for the eight-hour experimental period, either after a twenty-four hour fast or within thirty minutes after being fed one-half their daily ration of dog biscuit.[3] The secretion was collected at one or two-hour

[1] This investigation was supported by a grant from the National Research Council.

[2] The data in this paper are taken from a thesis presented by Kathryn Ferguson Fink to the Graduate School, University of Rochester, in partial fulfillment of the requirements for the degree Doctor of Philosophy, 1943.

[3] Purina dog chow.

intervals by means of soft rubber catheters inserted into the fistulae. The volume was recorded in milliliters, and the juice was then homogenized by an electric stirrer.

The concentrations of amylase-maltase complex, invertase and peptidase were determined by modifications of the methods described earlier (7). A twenty-four hour incubation period was used. The substrates consisted of a 4 per cent starch solution, a 5 per cent sucrose solution, and a 5 per cent Difco-Bacto Peptone solution. If the quantity of secretion were sufficient, a macro method was employed for the carbohydrate-splitting enzymes using 1.0 ml. of intestinal juice incubated with 50 ml. of the substrate. The phosphatase activity of the intestinal juice was determined by a modification of the method of Bodansky (8). Ten milliliters of buffered sodium β-glycerophosphate and 0.1 ml. of intestinal juice were incubated at 38°C. for exactly thirty minutes. At the end of this time, 10 ml. of 10 per cent trichloracetic acid were added, the contents mixed and filtered. An aliquot of the filtrate was analyzed for inorganic phosphorus by the method of Fiske and Subbarow (9) and the intensity of the color was measured in a Klett-Summerson photoelectric colorimeter. The concentration of inorganic phosphorus per milliliter of juice was subtracted from the total amount of inorganic phosphorus measured after the hydrolysis of the substrate to obtain a true phosphatase activity value. The concentration of mucoprotein was determined by measuring the hexose reducing power after acid hydrolysis of the juice. A modification of the method of Anderson, Fogelson and Farmer (10) was used. Thiocyanate was determined photometrically by the method of Lavietes et al. (11). Total CO_2 was determined according to Van Slyke and Neill (12).

The concentrations of the carbohydrate-splitting enzymes, peptidase and phosphatase, were expressed as milligrams of reducing sugar, amino nitrogen and inorganic phosphorus, respectively, which were released by enzymatic hydrolysis of the substrate under the conditions used by 1 ml. of succus entericus. The concentration of mucoprotein was expressed as milligrams of reducing sugar produced by acid hydrolysis of 1 ml. of intestinal juice. The concentrations of thiocyanate and total CO_2 were expressed as milliequivalents per liter.

RESULTS. *Colonic secretion.* A summary of the data obtained on the secretion of the colon is given in table 1. In these experiments, the secretion was collected and the volume determined at two-hour intervals, but the analyses were made on the total amount of juice obtained over eight hours, since the amount of fluid obtained in two hours was usually insufficient to permit complete enzyme and mucoprotein determinations. The scanty flow of fluid from the colonic loop is expected since water is absorbed in large quantities from this portion of the intestine. Not only was the volume of colonic juice small, but the concentrations of invertase and peptidase were very low as compared with the secretions obtained from the jejunum and ileum. In contrast, the concentration of mucoprotein was greater in the secretion of the colon than in the upper portions of the gastrointestinal tract. The secretion of the colon appeared to be unaffected by the administration of food.

One and one-half grams of sodium thiocyanate dissolved in 100 ml. of distilled water were given to the animals by stomach tube at the beginning of the experiment, and the rest of the procedure was the same as that for the control experiments. The data in table 1 demonstrate a tremendous increase in secretion following the administration of thiocyanate. There was approximately a fourfold increase in the quantity of secretion and total enzyme and mucoprotein production in the thiocyanate experiments on dog 3. The concentrations found in these two experiments are all in the range of the control values with the exception of the mucoprotein, the concentration of which is about one-half that of the control figures. In twelve control experiments on dog 4, no more than 0.2 ml. of juice was ever collected in eight hours, and this was insufficient to obtain control values for enzyme activity. In the two experiments with thiocyanate, the volume was increased to 3.8 and 3.6 ml., thus permitting complete enzyme

TABLE 1

Total eight-hour production of colonic secretion as influenced by sodium thiocyanate

	VOLUME	CONCENTRATION PER ML.				TOTAL (VOLUME × CONCENTRATION)			
		Amylase	Invertase	Peptidase	Mucoprotein	Amylase	Invertase	Peptidase	Mucoprotein
Dog 3									
	ml.	*mgm. glucose*	*mgm. invert sugar*	*mgm. amino-N*	*mgm. reducing sugar*	*mgm. glucose*	*mgm. invert sugar*	*mgm. amino-N*	*mgm. reducing sugar*
Controls									
Fasted (5)	2.45	149	47.0	6.49	4.33	366	119	15.9	10.3
Fed (7)	2.25	181	40.5	7.33	5.55	407	91	16.5	7.6
NaSCN	9.0	219	37.3	6.81	2.39	1976	336	61.3	21.5
NaSCN	10.0	223	35.2	6.75	2.37	2230	352	67.5	23.7
Dog 4									
Controls (12)	Did not secrete more than 0.2 ml. of juice in 8 hours								
NaSCN	3.8	680	82.1	10.7	4.84	2583	312	40.7	18.4
NaSCN	3.6	693	85.8	16.3	4.61	2496	302	58.6	16.6

analyses. With both dogs, the volume was greatest during the first two hours after the salt was given.

Ileal secretion. One and one-half grams of sodium thiocyanate in 100 ml. of distilled water were given dogs 1 and 2, which were animals bearing denervated ileal transplants, just after the succus entericus secreted during the first two hours had been collected. The run was then continued for six hours. The production for the first two hours, before thiocyanate was given, was used as a control for each of these experiments. Figure 1 represents a typical experiment. There was a marked increase in the volume, enzyme and mucoprotein production, and with the exception of invertase, the peak was reached at the second hour after thiocyanate administration. The thiocyanate concentration in the secretion had nearly reached its maximum by the second hour. The concentration of CO_2 in the juice was not significantly altered by thiocyanate. Since the

concentrations of enzymes and mucoprotein were not greatly altered, the large increase in the secretion of these substances was accounted for by the increase in the volume of juice. It appears that the effect of sodium thiocyanate may be to excite the glands to produce more than the usual amount of a normal secretion.

Table 2 is a summary of the experiments on ileal secretion. The ratios for the volume and enzymes in the thiocyanate experiments show a definite increase over the controls values obtained.

Jejunal secretion. The usual quantity of sodium thiocyanate was given by stomach tube to the dogs with Thiry-Vella fistulae of the jejunum at the end of

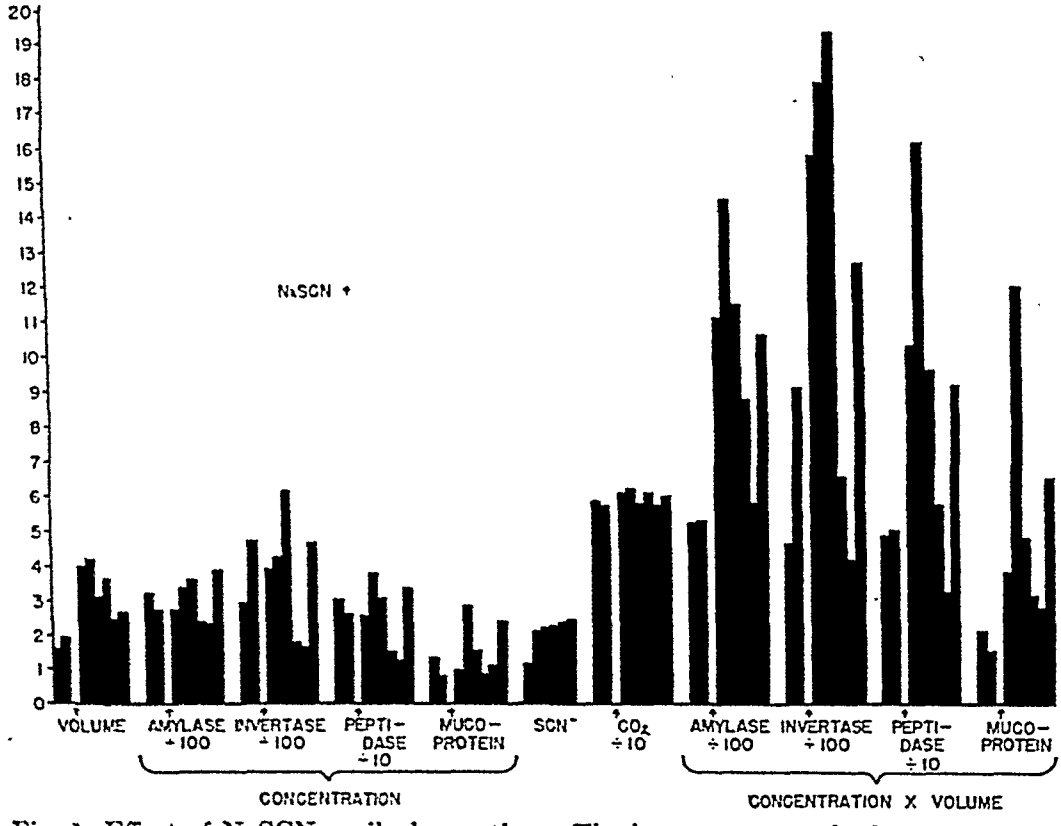

Fig. 1. Effect of NaSCN on ileal secretion. The bars represent the hourly values for volume, concentration of enzymes, mucoprotein, thiocyanate and CO_2 and the total amount of enzymes and mucoprotein over an eight hour period. Units are defined in the section on methods. The arrows indicate the time of administration of sodium thiocyanate.

the second hour of collection. The data on these animals were calculated in the same manner as that employed with the ileal transplant dogs, using the ratio of the production during the last six hours to the production during the first two hours. With both dogs, 12 control experiments, 6 after fasting and 6 after feeding, were done. The overall control ratios, together with the ratios in the thiocyanate experiments, are given in table 3. The increase in secretion as a result of the administration of thiocyanate is obvious. The increased production was greatest during the first two hours after the salt was given.

Duodenal secretion. No catheter or any sort of mechanical stimulation was

used to collect the duodenal secretion, inasmuch as the fistula was only about 3 cm. long, which is insufficient to keep a catheter in place. The secretion was extremely slimy and sticky and usually colorless. Some fifty determinations

TABLE 2

Ileal secretion expressed as ratio: $\frac{\textit{Total production in last six hours}}{\textit{Total production in first two hours}}$

	VOLUME	AMYLASE	INVERTASE	PEPTIDASE	MUCOPROTEIN
Dog 1					
Controls:					
Fasted (7)	2.22	2.80	2.99	1.98	
Fed (8)	2.81	2.44	2.71	2.01	
NaSCN	6.60	5.17			
NaSCN	5.77	5.93	5.54	5.46	9.14
NaSCN	5.00	8.29	5.62	5.44	7.03
Dog 2					
Controls:					
Fasted (7)	3.25	3.18	3.46	3.80	
Fed (5)	3.14	2.97	2.31	4.12	
NaSCN	12.30	9.55			
NaSCN	9.86	6.40	10.05	9.80	
NaSCN	8.49	4.38	4.24	6.87	3.96

TABLE 3

Jejunal secretion expressed as ratio: $\frac{\textit{Total production in last six hours}}{\textit{Total production in first two hours}}$

	VOLUME	AMYLASE	INVERTASE	PEPTIDASE	MUCOPROTEIN	PHOSPHATASE
Dog 7						
Controls:						
Fasted (6)	2.36	1.87	2.41	2.92	2.47	2.68
Fed (6)	1.74	1.84	1.18	2.05	1.71	2.00
NaSCN	4.70	3.36	6.31	3.67	4.14	3.30
NaSCN	9.08	5.17	4.14	5.11	4.27	2.22
Dog 8						
Controls:						
Fasted (6)	2.37	1.56	1.17	2.30	2.33	1.90
Fed (6)	2.54	1.42	1.09	1.73	1.97	1.56
NaSCN	6.80	4.78	4.03	5.15	5.16	4.04
NaSCN	6.79	4.55	5.38	4.74	6.67	4.47

of the pH of the juice were made, and they ranged between 7.1 and 8.8 with most of them falling around 8.0. Wright et al. (13) found amylase and enterokinase present in duodenal juice, but tests for dipeptidase and polypeptidase were

nearly always negative. The secretion from the dog reported here consistently contained peptidase, although the concentration was very low. Invertase was found only occasionally, and then in traces, which is in agreement with the findings of Wright. The volume of duodenal secretion found is in the same range as that reported by other investigators (13, 14).

TABLE 4

Hourly production of duodenal secretion as influenced by NaSCN

(Dog 5)

HOUR	VOLUME	CONCN. PER ML.		TOTAL (VOLUME × CONCN.)		SCN CONCN. IN JUICE
		Amylase	Peptidase	Amylase	Peptidase	
	ml.	*mgm. glucose*	*mgm. amino-N*	*mgm. glucose*	*mgm. amino-N*	*m.eq./l.*
1	2.3	45.0	0.8	104	1.8	
2	0.1					
3	0.0	15.0	1.9	4.5	0.6	
4	0.2					
1½ grams NaSCN given						
5	2.2	82.9	3.0	182	6.6	2.44
6	1.0	347	6.9	347	6.9	4.21
7	0					
8	0					
1	0.28	158	9.0	44	2.5	
2	0.4	107	7.0	43	2.8	
3	1.0	168	9.0	168	9.0	
1½ grams NaSCN given						
4	1.6	186	20.0	297	32.0	5.52
5	0.7	166	11.1	116	7.8	6.54
6	0.5	158	19.0	79	9.5	6.47
7	0.3	161	17.1	48	5.1	
8	0.3	156	13.0	47	3.9	
1	3.2	59	0.8	188	2.6	
2	0.5	61	3.0	31	1.5	
3	0.5	36	2.5	18	1.3	
1½ grams NaSCN given						
4	0.8	64	4.7	51	3.8	1.50
5	1.8	71	4.6	128	8.3	6.17
6	2.0	51	0.19	102	3.8	5.67
7	0.2	82	7.4	49	4.4	3.73
8	0.4					

In three experiments, sodium thiocyanate was given the duodenal fistula-bearing dog; the data are shown in table 4. In the hour or two following the administration of the salt, there was a slight rise in the volume and total amylase and peptidase production as compared with the output during the two hours previous to the administration of thiocyanate.

In vitro studies. A series of experiments using the Warburg technique showed that thiocyanate in a concentration of 6 millimols per liter, which was the maxi-

mum concentration found in the secretion in the *in vivo* experiments, had no significant effect on the respiration of intestinal mucosa. This would suggest that the increased secretory activity occasioned by thiocyanate is accomplished at a small cost of energy. The failure to demonstrate an increased respiration of the mucosa does not, of course, rule out a possible respiratory effect *in vivo*.

The addition of thiocyanate to normal juice in concentrations found in the juice when the drug was given orally, did not affect enzyme activity.

Discussion. The increase in intestinal secretion following the administration of sodium thiocyanate is in marked contrast to the effect of this drug on gastric and pancreatic secretions. Davenport (1) found an inhibition of gastric secretion, and Tucker and Ball (2) found thiocyanate injections had no effect on the rate or bicarbonate content of pancreatic secretion, unless very high dosages were given. Then there was a general toxic effect and a decrease in the rate of secretion. Thus, different digestive secretions are differently affected by thiocyanate. It appears that the results may be in accord with the reverse of the gradient theory of Alvarez (15)—an inhibition in the stomach, a slight stimulation in the duodenum, a greater stimulation in the jejunum and ileum, and a still greater effect in the colon.

The mode of action of sodium thiocyanate on the gastrointestinal secretions is not known. Several possible mechanisms of action might be mentioned: 1. It may act by stimulating the production of enterocrinin and enterogastrone in the gut; the former would increase intestinal secretion and the latter would inhibit gastric secretion. 2. It may act directly on the secretory glands, inhibiting those in the stomach, and stimulating those in the intestine. 3. Little is known about the influence of the intrinsic nerves on intestinal secretion, but there is a possibility that thiocyanate produced its effect by action on these nerves. 4. It may have produced its effect by blocking an inhibition which may normally be present in the control of secretion.

SUMMARY

Sodium thiocyanate, given by stomach tube in a one and one-half gram dose, increases the rate of intestinal secretion. The volume, total enzymes, and mucoprotein are affected. It appears that the results may be in accord with the reverse of Alvarez gradient theory—an inhibition in the stomach, a slight stimulation in the duodenum, a greater stimulation in the jejunum and ileum, and a still greater effect in the colon.

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THE EFFECT OF THE THYROID ON JEJUNAL SECRETION IN THE DOG[1]

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Received for publication April 7, 1944

Hyperthyroidism as it is observed in the clinic is frequently accompanied by gastro-intestinal symptoms, the most common of which are increased desire for food, and diarrhea. An idea of the frequency of these gastro-intestinal symptoms may be obtained from the work of Tinker (1) who reported digestive disturbances in 1415 of 4127 cases of goiter. He believes that among the most dangerous symptoms encountered in toxic goiter cases are the digestive symptoms. Although it is accepted that the thyroid gland exerts an effect on the digestive tract, the fundamental changes are not well understood. Any study of these changes can be divided into two main groups, namely, secretory activity and motor activity. It is the purpose of this study to investigate the influence of the thyroid gland on the secretion of the jejunum in the hope that some physiological information can be added to the knowledge of the clinical picture of thyroid diseases.

The dogs used in this study had Thiry-Vella fistulae of the jejunum. The animals were set up in stalls for an eight-hour experimental period, either after a twenty-four hour fast or within thirty minutes after being fed one-half their daily ration of dog biscuit.[3] The secretion was collected every two hours by means of soft rubber catheters inserted into the fistulae. The volume was recorded in milliliters, and the juice was then homogenized by an electric stirrer. The methods used for determination of mucoprotein and the various enzymes and the units have been described by Fink and Nasset (2). The basal metabolism was obtained by the Tissot-Haldane procedure. A special head piece described by Kochakian and Murlin (3) was used. Surface area was calculated according to the Cowgill and Drabkin (4) formula for dogs.

In general, two or three eight-hour secretion experiments were done each week, and the basal metabolism was measured once a week between the first and second secretion runs.

Squibb's racemic thyroxin was given subcutaneously. One milligram per kgm. body weight was given in the first week of the thyroxin periods, $1\frac{1}{2}$ mgm. per kgm. the second week, and 2 mgm. per kgm. per week for the remainder of the periods.

[1] This investigation was supported by a grant from the Committee on Research in Endocrinology of the National Research Council.

[2] The data in this paper are taken from a thesis presented by Kathryn Ferguson Fink to the Graduate School, University of Rochester, in partial fulfillment of the requirements for the degree, Doctor of Philosophy, 1943.

[3] Purina Chow.

The parathyroids were left intact when thyroidectomy was performed as evidenced by normal serum calcium thereafter.

Statistical analysis of the data was performed by the use of the Fisher "t" test (5).

RESULTS. A brief summary of the data obtained in the basal metabolism studies for dog 1 is given in table 1. The results on dog 2 followed the same general trend. In both animals there was a slight decrease in weight during the periods in which thyroxin was given, and a gain in weight after thyroidectomy when no thyroxin was given. The calories per hour derived from protein were calculated from the urinary nitrogen excretion, and they rose somewhat in the thyroxin periods, but there was no marked decrease resulting from thyroidectomy. This is in accord with the work of Janney (6) who found that thyroidectomy has little effect on the urinary nitrogen of the dog. The average of the determinations of total calories per square meter of body surface per hour during control I and control II, which is considered here as the normal level of metabolism, was 39.2 for dog 1. It is of interest that Kitchen (7) obtained an average of 39.2 calories per square meter per hour in 254 observations on 13 dogs. The figures in the last column were calculated on the basis of the average calories obtained during the first two control periods as 100 per cent. As was to be expected, the energy metabolism rose above normal when thyroxin was given and fell after thyroidectomy. The same amount of thyroxin per unit of body weight was given per week to the animals in the thyroxin I period as in the thyroxin II period but the per cent increase in metabolism from the previous period was greater when the animals were thyroidectomized. This increased sensitivity of thyroidectomized animals to thyroxin has been reported by Fleischmann et al. (8) in the rabbit, by Smith et al. (9) in the rat, and by Thompson et al. (10) in myxedematous patients.

The results on jejunal secretion are shown in table 2 for dog 1.

Thyroxin injections were given dog 1 over a period of three weeks following the control I period. The metabolism rose to a maximum of 141 per cent. During the period of thyroxin treatment, the average two-hour values for volume, amylase, invertase, peptidase and mucoprotein were elevated to approximately 200 per cent of the controls, both after fasting and feeding. Statistically these increases were highly significant. The P value in every case indicated that the probability of such results occurring by chance was less than 1 per cent. Although phosphatase increased 14 per cent in the fasting studies and 22 per cent in the feeding studies, these increases were not statistically significant. This difference with phosphatase recurred all through the work but no satisfactory explanation can be given.

The control II period was begun two weeks after the last thyroxin injection and extended over nine weeks. In both fasting and feeding experiments, all the factors of the jejunal secretion that were determined were lower than those in the thyroxin I period with the exception of phosphatase in which there was a slight but not significant increase. The changes in the other components of the secretion were all statistically significant decreases, with the exception of amylase

and invertase in the fasted state, and amylase in the fed state. In these three cases the values in the last part of the period were in the range of those in the control I, and would probably have shown significant decreases had the control II period been extended for a longer time. All the components of secretion which were investigated were higher in control II than in control I, and these changes were all highly significant statistically. The most obvious explanation is that the thyroxin treatment exerted a prolonged effect on secretion in spite of the fact that the basal metabolism throughout the control II period was comparable to that in control I.

Following the control II period the animal was thyroidectomized and studied in this state for six months. As a result of the removal of the thyroid, the basal metabolism fell to an average of 85 per cent of normal. All factors observed in secretion were lower in the thyroidectomy period than in control II. These decreases were all statistically significant, with the exception of mucoprotein in the experiments in which the dog was fed. From table 1 it can be seen that the values in the thyroidectomy period compare quite closely with those in control I. This indicates that thyroidectomy did not result in a subnormal production of intestinal secretion and adds further evidence that the thyroxin injections had a prolonged effect upon secretion which resulted in high values during control II.

Thyroxin was again given dog 1, this time in the thyroidectomized state. The hormone injections were given for five weeks, and the basal metabolism rose to a maximum of 154 per cent of the normal. All the various constituents of the juice that were followed increased significantly during the thyroxin II period over the previous thyroidectomy period, with the exception of phosphatase, which decreased but not significantly.

The control III period was begun three weeks after the last thyroxin injection was given the thyroidectomized animal, and continued for six weeks. During the first two weeks of this period, there seemed to be a slight hang-over effect of thyroxin on the basal metabolism. The metabolism was 97 and 90 per cent of the normal in two determinations in these first two weeks, but the fourth week it was down to 81 per cent of normal, which is comparable to that in the thyroidectomy period. In the control III period, all the components of secretion decreased significantly from the values obtained in the previous thyroxin period.

The data on jejunal secretion for dog 2 are shown in table 3. The result of the experiments in which the animal was fed follow the same general trend as dog 1. The secretion values were increased during the periods of thyroxin administration. A "hang-over" effect of thyroxin was observed in the control II period. The secretion of fluid and enzymes was decreased during the thyroidectomy period, but the levels were not subnormal. The values obtained during the control III period were about the same as those during the control I period.

The results in the experiments in which dog 2 was fasted differ somewhat from those of dog 1. Although the data from dog 2 are as complete as for dog 1, and all the secretion values observed increased during the thyroxin I period, only the increases in volume, peptidase and mucoprotein were statistically significant.

The values showed an increase in the control II period and there was little change from the latter in the thyroidectomy period. No satisfactory explanation for

TABLE 1

Energy metabolism data

(Dog 1)

	DATE	WT. OF ANIMAL	CAL./HOUR FROM PROTEIN	TOTAL CAL./SQ.M./HOUR	PER CENT OF NORMAL METABOLISM
		lbs.			
Control I	1-17-41	31	2.2	37.3	95
	1-24-41	31	3.5	39.8	102
Thyroxin I	1-28-41	30	4.3	44.3	113
	2- 5-41	30	5.2	45.6	116
	2-11-41	29	6.2	55.1	141
Control II	2-25-41	29	5.0	41.1	105
	3- 4-41	29	3.6	38.1	97
	3-11-41	29	3.6	39.9	102
	3-18-41	29	3.7	38.4	98
	4- 1-41	29	4.6	41.0	105
	4-23-41	27	4.8	38.3	98
Thyroidectomy	5-20-41	28	4.1	32.8	84
	5-27-41	29	3.2	31.5	80
	6-17-41	30	4.5	32.7	83
	6-24-41	31	3.9	34.2	87
	7- 1-41	31	4.4	33.4	85
	7- 8-41	31	4.6	31.3	80
	7-15-41	31	4.8	34.0	87
	7-22-41	32	4.6	35.4	90
	8-22-41	34	4.5	33.3	85
	8-29-41	35	3.9	32.5	83
	10-29-41	35	3.6	33.0	84
	12- 6-41	35	4.2	34.1	87
	12-12-41	35	4.0	33.6	86
	1- 6-42	33	3.7	34.2	87
Thyroxin II	2-17-42	32	7.3	49.9	127
	2-24-42	32	7.0	57.1	146
	3-10-42	30	5.8	60.3	154
	3-17-42	32	5.8	50.8	130
	3-24-42	30	6.0	59.0	151
Control III	4-14-42	31	3.7	38.2	97
	4-21-42	31	3.3	35.3	90
	5- 5-42	33	3.3	34.5	88
	5-12-42	35	2.8	31.7	81

these differences between the two dogs is available. Increases in the thyroxin II period were statistically significant when compared with the control III period.

Discussion. The effect of thyroxin in accelerating intestinal secretion is in contrast with its action on gastric secretion. Truesdell (11) reported that experimental hyperthyroidism in dogs resulted in a decrease in rate of gastric secretion and in the free and total acidity.

Fetter and Carlson (12) studied the effect of experimental hyperthyroidism on gastro-intestinal motility of dogs. Their work is of particular interest here because of the "hangover" effect of thyroxin. Six dogs were used, and the gastric motility of all the animals increased during the period of induced hyperthyroidism. In four of the animals, after the thyroid feeding was discontinued, the motility decreased below that observed in the hyperthyroid state, but it did not drop to the normal level except occasionally. The basal metabolism was

TABLE 2

Average two-hour production of jejunal secretion in each period

(Dog 1)

	VOLUME	AMYLASE	INVERTASE	PEPTIDASE	MUCOPROTEIN	PHOSPHATASE	NO. OF EXPERIMENTS	APPROXIMATE DURATION
	ml.	*mgm. glucose*	*mgm. invert sugar*	*mgm. amino-N*	*mgm. reducing sugar*	*mgm. inorganic P*		*weeks*
Fasted:								
Control I	15.9	1626	3193	302	20.7	298	28	5
Thyroxin I	49.9	3289	7747	697	41.7	339	16	3
Control II	34.2	2882	6287	508	33.1	367	22	9
Thyroidectomy	20.3	1400	2784	372	26.7	246	48	24
Thyroxin II	43.6	3754	5730		43.3	239	28	5
Control III	28.2	1728	3891		31.4	208	24	6
Fed:								
Control I	24.6	1991	3913	367	24.7	288	20	5
Thyroxin I	58.6	3543	8583	651	43.5	350	16	3
Control II	44.7	2972	6238	549	37.0	389	32	9
Thyroidectomy	26.7	1836	3410	452	33.0	293	44	24
Thyroxin II	48.3	3538	4949		43.8	235	28	5
Control III	26.9	2040	3135		28.2	189	24	6

studied on one animal, and it fell from +50 per cent to normal at the end of the first week after thyroid feeding was discontinued and remained there. The gastric motility, however, never returned to normal during the two months that contractions were followed after stopping the thyroxin treatment. They could offer no satisfactory explanation for the prolonged effect of thyroxin. They also found that the emptying time of the stomach was decreased, and the intestinal motility was increased during experimental hyperthyroidism.

In a further investigation Fetter and Carlson (13) found that thyroxin increased hunger contractions and the speed with which a barium meal passes through the digestive tract in vagotomized dogs. They concluded that the influence of thyroxin on gastrointestinal motility is largely, if not entirely, in-

dependent of the gastrointestinal vagus mechanism. Morrison and Feldman (14) also reported that thyroid administration to normal and vagotomized dogs increased the gastrointestinal motility.

Althausen and Stockholm (15) found that induced hyperthyroidism in rats markedly increased the absorption of glucose, galactose, xylose and oleic acid from the digestive tract, and also increased motility.

The influence of experimental hyperthyroidism and thyroidectomy on several enzymes has been reported in the literature. Klein (16) found the concentration of d-amino acid oxidase in rat liver was increased by feeding thyroid and decreased by thyroidectomy, but the same enzyme in the kidney was unaffected. Scoz and Marangoni (17) reported that thyroxin increased the plasma phos-

TABLE 3

Average two-hour production of jejunal secretion in each period

(Dog 2)

	VOLUME	AMYLASE	INVERTASE	PEPTIDASE	MUCOPROTEIN	PHOSPHATASE	NO. OF EXPERIMENTS	APPROXIMATE DURATION
	ml.	*mgm. glucose*	*mgm. invert sugar*	*mgm. amino-N*	*mgm. reducing sugar*	*mgm. inorganic P*		*weeks*
Fasted								
Control I	4.56	848	1185	124	13.5	149	27	5
Thyroxin I	8.53	974	1224	206	19.9	152	32	5
Control II	9.34	1027	1642	240	19.4	218	28	8
Thyroidectomy	9.32	1111	1410	184	21.3	142	52	24
Thyroxin II	10.6	1060	1476		17.2	110	32	6
Control III	7.4	757	1054		13.9	110	28	6
Fed:								
Control I	5.68	730	991	118	11.5	130	24	5
Thyroxin I	18.9	1494	1982	260	23.3	187	28	5
Control II	14.1	1419	1399	209	17.7	188	32	8
Thyroidectomy	9.93	931	925	138	15.9	89.8	40	24
Thyroxin II	15.2	1077	1327		17.4	110	32	6
Control III	8.9	715	1064		13.3	113	28	6

phatase of dogs to double or triple normal values. Castagna (18) noted that the blood catalase in dogs decreased to about 50–70 per cent of the original values after thyroidectomy. Cohen and Gerard (19) found evidence which indicates that the absolute concentration of dehydrogenases and oxidases is greater in the hyperthyroid than in the normal rat brain.

Thus, it has been found that the concentration of several enzyme systems is increased in hyperthyroidism or decreased by thyroidectomy, and that gastric secretion and acidity are decreased in hyperthyroidism, while gastrointestinal motility and absorption from the gut are increased. The results reported here show that the production of intestinal secretion both as regards volume and amount of several enzymes is increased in hyperthyroidism.

The mechanism responsible for the effect of thyroxin on the secretion of the jejunum is not obvious. There are several possibilities that may be involved in this thyroid-intestinal relationship. *1.* The increased blood flow in hyperthyroidism (20) may result in an increased secretion of the gut. *2.* Thyroxin may have a direct effect on the secretory cells of the intestine. *3.* The alterations in intermediary metabolism in hyperthyroidism may secondarily affect the activity of the secretory cells of the jejunum. *4.* The effect on secretion may be a consequence of the action of thyroxin on other endocrine substances. One possibility is the stimulation of the production of enterocrinin, which is known to accelerate the secretion of the gut (21). Timme (22) has pointed out that the thyroid rarely functions alone, and any disturbance of this gland always induces a change in the function of other glands. *5.* The action of thyroxin on the intestine may be through the nerves. Gerard and McIntyre (23) found that the oxygen consumption of the vagus taken from hyperthyroid dogs was increased about 25 per cent. Possibly the increased activity of the nerves could influence the secretory cells. The evidence at hand does not favor this theory. Satisfactory evidence that vagal stimulation increases the secretion of the jejunum has not been obtained, and the sympathetics have an inhibitory action on intestinal secretion. Furthermore, there is a great deal of evidence that the action of thyroxin is not primarily through the nerves (13, 24, 25, 26). *6.* The increased energy metabolism in hyperthyroidism may secondarily influence secretion. The oxygen consumption of all the tissues that have been studied from hyperthyroid animals is increased, with the exception of the thyroid gland. As a consequence of the elevated metabolism, it is conceivable that the processes of the intestine are speeded up—secretion, absorption and motility. If, however, the increased intestinal secretion were a result of the increased metabolism, it would be difficult to understand why gastric secretion should be depressed in hyperthyroidism. It would also be difficult to explain the prolonged action of the thyroxin on jejunal secretion into the control II period when the basal metabolism was normal, and to account for the normal secretion after thyroidectomy when the energy metabolism was deceased.

SUMMARY

1. Administration of thyroxin to dogs, in doses sufficient to elevate the basal metabolism to hyperthyroid levels, resulted in a significant increase in the rate of jejunal secretion.

2. Thyroxin exerted a prolonged effect on jejunal secretion for several weeks after the basal metabolism had returned to normal.

3. Thyroidectomy did not result in a subnormal rate of jejunal secretion in the dog.

4. In general, the total production of mucoprotein and all enzymes but phosphatase changed in a parallel fashion with the volume of secretion.

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ADRENALECTOMY, GONADECTOMY AND THE INSULIN CONTENT OF THE PANCREAS

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Received for publication April 12, 1944

It was reported previously (Best, Haist and Ridout, 1939) that fasting or the feeding of fat led to a reduction in the insulin content of the pancreas in rats. This reduction seemed to be related in large part to the deficiency of carbohydrate or carbohydrate-forming substances in the diet. At the time the first report was made it was not known whether or not the lowering of the insulin content of the pancreas might be brought about indirectly through an influence of these procedures on other endocrine glands such as the pituitary or adrenals. Since then it has been shown that removal of the pituitary gland does not prevent the reduction in the insulin content of the pancreas which results from fat-feeding in rats (Haist and Best, 1940; Haist, 1940).

In the present report data are presented which indicate that this effect of fat-feeding on the insulin content of the pancreas can still be obtained in the absence of the adrenal glands[1] and in the absence of the gonads.

MATERIAL AND METHODS. In the experiments involving adrenalectomy, female rats of the Wistar strain were used in all but one of the tests. The pancreatic tissue from a group of 10 rats was used for the insulin extraction, and the potency of the solution was determined by the mouse method of assay. These procedures have been outlined in a previous report (Best, Haist and Ridout).

RESULTS. *Insulin content of pancreas and body weight.* In the present series of experiments the rats were of light weight. Confirming the observations of Griffiths (1941) it was found that the lighter rats had a smaller insulin content of pancreas than heavier ones. This is illustrated in figure 1, which collects some values for the insulin content of the pancreas in male and female rats of different weights. It will be evident that the pancreases of the smaller rats contain less total insulin than the pancreases of the heavier rats.

The effect of adrenalectomy. Six groups of 10 rats were bilaterally adrenalectomized, fed as much as they would eat of a complete diet and given 1 per cent NaCl to drink instead of water. Six control groups were given the same diet and the same caloric intake as the adrenalectomized groups and were also given 1 per cent NaCl to drink. The rats were sacrificed and the pancreases taken from 3 to 10 days following adrenalectomy. The insulin assays on the pancreases of these animals are shown in table 1. It is evident that the values for the insulin content of the pancreases in the adrenalectomized animals receiving NaCl do not differ significantly from those obtained in the pair-fed

[1] A preliminary report of part of this work appeared in this Journal, 1941, 133: P 310.

control rats. Table 2 shows that the average initial weight of the adrenalectomized animals was 126 grams. The average insulin content was 12.5 units per group of 10 rats. The average initial weight of the pair-fed control rats was 127 grams and the insulin content was 12.4 units per group. The average insulin content for 6 groups of normal rats weighing 122 grams, approximately the same weight, was 12.9 units per group. From these results it would appear that adrenalectomy leads to no significant change in the insulin content of the rat pancreas.

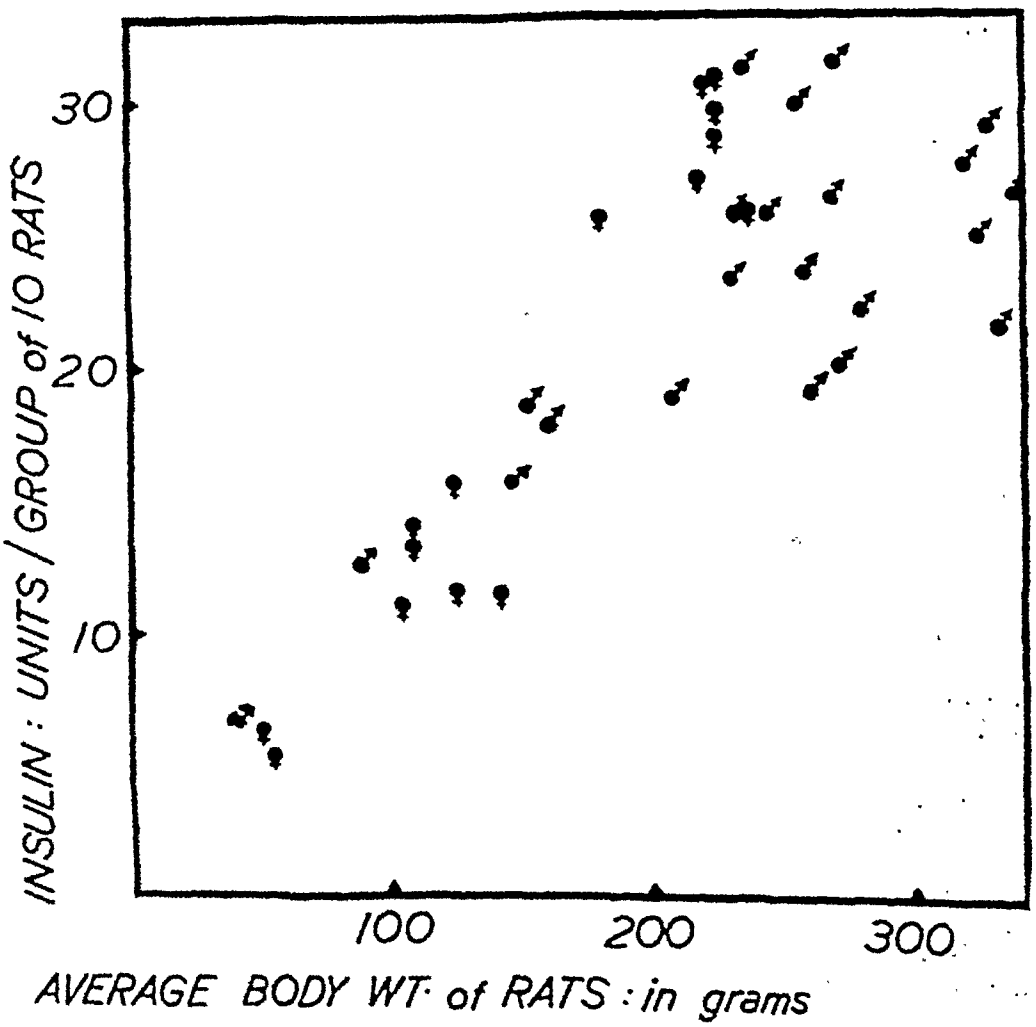

Fig. 1

Five groups of rats were adrenalectomized and then fed a diet very rich in fat for 7 days (item 5, table 2). They received 1 per cent NaCl to drink. Three control groups with intact adrenals were treated in the same fashion and received the same caloric intake (item 6, table 2). It will be evident that the feeding of fat led to a great reduction in the insulin content of the pancreas in both the control and adrenalectomized groups. There is, with the fat-feeding, an undernutrition effect also, since animals on the fat diet have a lower caloric intake than those receiving the balanced ration. The results indicate that, as far as

the insulin content of the pancreas is concerned, the adrenalectomized animals receiving NaCl respond to this treatment in a manner similar to the normal. The table also shows that, following fat-feeding, the insulin content of the pancreases of adrenalectomized rats can be restored to normal by the administration of a balanced diet.

TABLE 1

The effect of adrenalectomy on the insulin content of pancreas

NUMBER OF DAYS AFTER ADRENALECTOMY	UNITS OF INSULIN PER GROUP OF 10 RATS		UNITS OF INSULIN PER 1000 G. INITIAL WEIGHT OF RATS	
	Adrenalectomized	Controls pair-fed	Adrenalectomized	Controls pair-fed
3	14.0	12.0	11.6	9.4
10	14.7	14.5	12.1	11.9
7	11.4	12.0	9.8	10.4
7	11.8	10.7	10.0	9.1
7	10.7	11.7	7.3	8.0
7	12.1	13.2	9.1	9.8
Average	12.5	12.4	10.0	9.8

TABLE 2

GROUP	NO. OF GROUPS TESTED	AVEREGE BODY WEIGHT OF RAT		AVERAGE INSULIN UNITS PER GROUP 10 RATS	AVERAGE INSULIN U./1000 G. INITIAL BODY WEIGHT
		Initial	Final		
1. Normal rats.....	6		122	12.9	10.6
2. Adrenalectomized fed ad lib (1% NaCl).....	6	126	136	12.5	10.0
3. Controls: paired-fed with 2 (1% NaCl).....	6	127	128	12.4	9.8
4. Controls: paired-fed (no NaCl).....	1	118	114	12.7	10.8
5. Adrenalectomized fed fat (1% NaCl)..	5	144	106	2.2	1.5
6. Controls: fed fat. Paired with 5 (1% NaCl).....	3	147	115	3.1	2.1
7. Adrenalectomized fed fat then fed balanced diet (1% NaCl).....	1	170	157	16.6	9.8
8. Controls: paired-fed with 7. Fed fat, then balanced diet (1% NaCl).....	1	161	154	16.5	10.2

Table 3 indicates that removal of the ovaries in female rats or removal of the testes in males, does not quickly alter the insulin content of the pancreas (14–20 days). It shows too that the effect of fat-feeding on insulin content can be obtained in the absence of the gonads.

DISCUSSION AND CONCLUSIONS. Fraenkel-Conrat, Herring, Simpson and Evans (1942) found, in one experiment, that the pancreases of adrenalectomized rats contained more insulin than those of unoperated rats. It will be evident from the results presented that the removol of the adrenal glands led to no sig-

nificant change in the insulin content of the pancreas in our experiments. In some adrenalectomized groups the insulin content was higher and in some lower than normal. Fraenkel-Conrat et al. also reported that the administration of adrenal cortical extract did not elevate the insulin content. They did observe, however, that pituitary adrenocorticotropic preparations increased the insulin content of the rat pancreas, due, they thought, to contamination with lactogenic hormone. The finding of Ingle (1941) that glycosuria can be produced in the normal rat by the administration of 17-hydroxy-11-dehydrocorticosterone, and reports of Long, Katzin and Fry (1940) and of Ingle (1940) that the diabetic state of the partially depancreatized rat can be increased by certain adrenal steroids make it seem probable that while adrenal cortical extracts may have no demonstrable influence on the insulin content of the pancreas, yet if certain specific adrenal steroids were used in sufficient amounts some measurable effect on insulin content might be obtained. The fact that the reduction in insulin content of pancreas occasioned by fat-feeding can be obtained in the absence

TABLE 3

GROUP	AVERAGE BODY WEIGHT OF RAT		INSULIN CONTENT: UNITS PER GROUP OF 10 RATS	INSULIN: UNITS PER 1000 GRAMS INITIAL BODY WEIGHT
	Initial	Final		
Ovariectomized (20 days): normal diet	178	209	16.6	9.3
Controls: paired-fed, normal diet	178	185	14.5	8.1
Ovariectomized: normal diet 11 days, fat 7 days	178	171	7.1	4.0
Controls: paired-fed, normal diet 11 days, fat 7 days	178	167	6.8	3.8
Castrated (14 days): normal diet	249	251	21.8	8.8
Controls: paired-fed normal diet	249	236	20.1	8.1
Castrated: normal diet 5 days, fat 7 days	249	213	11.2	4.5

of the adrenal glands is additional argument, however, for a normal regulation of pancreatic insulin that is independent of the adrenal glands.

The experiments on gonadectomy, while few, indicate that the removal of the gonads in male and female rats has no appreciable effect on the insulin content of the pancreas. A definite elevation in insulin content of the pancreas in rats is reported to result from the administration of the oestrogens, oestrone (Marks and Young, 1940); oestradiol (Griffiths, Marks and Young, 1941; Funk, Chamelin, Wagreich and Harrow, 1941) and oestradiol dipropionate (Fraenkel-Conrat et al., 1941), oestriol (Griffiths, Marks and Young) and stilboestrol (Griffiths and Young, 1940, 1942; Griffiths, Marks and Young; Funk et al.). Hexoestrol elevated the pancreatic insulin content in the rabbit (Griffiths, 1942, 1943). The androgen, testosterone, did not induce an increase in pancreatic insulin in male rats (Marks and Young, 1940; Griffiths, Marks and Young; Funk et al.), and progesterone also had no insulin-increasing effect (Funk et al.). Alpha methyl stilbene, which is closely related to hexoestrol and stilboestrol, is non-oestrogenic yet it increases the insulin content of the pancreas in rabbits

(Griffiths, 1942, 1943), which fact prompted Griffiths to conclude that the insulin-increasing properties of hexoestrol and stilboestrol "have little to do with their oestrogenic properties." Fraenkel-Conrat et al. (1941) showed that the insulin-increasing effect of the oestrogen, oestradiol dipropionate, is not obtained in hypophysectomized rats and that implanted pituitaries from rats receiving oestrogens elevate the insulin content of the pancreas in the host while implanted pituitaries from control rats do not. They feel that this evidence favors the assumption that the insulin-increasing activity of oestrogens is mediated by the pituitary gland. As with the pituitary, the removal of the gonads does not prevent the reduction in insulin content of the pancreas which results from fat-feeding.

Under certain circumstances the insulin content of the pancreas is raised by the injection of extracts or materials obtained from endocrine glands. This does not mean that those endocrine structures have an important rôle in the normal regulation of pancreatic insulin. In some instances at least, the elevation in total content seems to be related to a multiplication of cells and an increase in islet volume (Richardson and Young, 1937; Marks and Young, 1939) and need not necessarily involve an increase in the concentration of insulin within each islet cell.

The finding that the insulin content of the pancreas is not greatly affected by adrenalectomy or gonadectomy and that the reduction in the insulin content of the pancreas that results from fat-feeding can still be obtained in the adrenalectomized or gonadectomized animals indicates that these endocrine structures are probably not fundamentally involved in the normal regulation of the insulin content of the pancreas. The pancreas appears to be able to regulate production and liberation according to the need for insulin in the absence of pituitary, adrenals or gonads.

SUMMARY

1. The insulin content of the pancreas in rats increases with age. The pancreases of smaller (younger) rats contain less total insulin than the pancreases of heavier (older) rats.

2. The insulin content of the pancreas in rats adrenalectomized for 3 to 10 days and maintained on sodium chloride does not differ significantly from that of control rats receiving the same caloric intake.

3. The insulin content of the pancreas in adrenalectomized rats is greatly reduced by the feeding of fat.

4. Following the reduction by fat-feeding, the insulin content can be restored to normal levels by the administration of a complete diet.

5. Removal of the ovaries in females or the testes in males does not lead to any significant change in the insulin content of the pancreas, as compared to controls receiving the same caloric intake.

6. The feeding of fat to gonadectomized rats leads to a reduction in the insulin content of the pancreas.

7. It is concluded that the production and liberation of insulin can be regulated in response to dietary changes in the absence of adrenals or of gonads.

It is a pleasure to thank Prof. C. H. Best, F.R.S., for his interest and help in this work.

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THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 141 JULY 1, 1944 No. 5

FACTORS RESPONSIBLE FOR THE INTESTINAL PHASE OF GASTRIC SECRETION[1]

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Received for publication March 21, 1944

Experiments performed in Pavlov's laboratory (Pavlov, 1910) suggested that the small intestine may be concerned in the mechanism responsible for gastric secretion. While the results were not always consistent, it was found that meat and meat extractives as well as many other substances excited the gastric glands when they were introduced into the small intestine. Ivy and his co-workers (Ivy and McIlvain, 1923; Ivy and Javois, 1924) studied this problem and concluded that part of the gastric secretion following the ingestion of a meal may be due to the presence of food in the intestine. The literature has been reviewed by Babkin (1928). From the data reported in the literature and the work done in his own laboratory, Babkin (1938) recently concluded that the "intestinal phase" of gastric secretion is a result of direct and indirect stimulation of the gastric glands by the end-products of digestion after their absorption. The present study was undertaken in the hope of obtaining additional information regarding the relative significance of and some of the factors involved in the so-called intestinal phase of gastric secretion.

METHODS. Eighty-five experiments were performed on 6 dogs. All animals were provided with gastric and duodenal fistulas by a method previously described (Thomas, 1941). In addition to the fistulas, one dog (dog B) had a Pavlov pouch, another dog (dog L) had a Thiry-Vella loop of high jejunum, while a third dog (dog M) had an ileal fistula below an end-to-side anastomosis. In this dog both vagi and both major splanchnic nerves had been severed.

Gastric juice was collected from the Pavlov pouch through a soft rubber tube inserted by way of a hard rubber cannula. The tube was kept permanently in place. Gastric juice was collected from the whole stomach by means of a soft rubber drain tube inserted through the gastric fistula.

Observations were begun about eighteen hours after the previous meal and only when the stomach was found to be in a state of secretory rest, unless the object of the experiment was such as to require a different procedure.

[1] Aided by a grant from John Wyeth and Bros., Inc.
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The substances whose secretagogue effect were to be tested were infused by gravity into the small intestine at a distance approximately 30 cm. below the pylorus by means of a soft rubber catheter inserted through the intestinal fistula. The rate of infusion was controlled by means of a burette periodically refilled from a Marriott bottle and was limited to a rate of 1.5 to 2 cc. per minute.

Reflux of the infused material into the stomach from the intestine generally was controlled adequately by draining the first part of the duodenum through the duodenal fistula by means of a perforated soft rubber drain tube as described elsewhere (Thomas, 1940). Experiments in which there was evidence of backflow of the stimulating substance into the stomach were discarded. Bile, however, frequently regurgitated into the stomach. Therefore, in some of the experiments, the duodenal drain tube was connected to a Wangensteen drainage bottle with a trap intervening. This device effectively prevented regurgitation of bile into the stomach or its accumulation in the intestine.

Results. We found, as have others, that following the introduction into the intestine, without special precautions, of substances such as ground meat or meat digests, a secretion of gastric juice was usually obtained. On the other hand, when bile was prevented from entering the stomach or accumulating in the intestine and only isotonic solutions were used, *little or no gastric secretion was evoked*. A typical result is shown in table 1A. Our experiments were therefore mainly directed toward an analysis of the various factors that were apparently responsible for the so-called intestinal phase of gastric secretion under the conditions of our experiments.

1. Presence of bile in the stomach. In seven experiments with the Pavlov pouch dog, in which products of protein digestion were instilled into the intestine, the bile entering the intestine was permitted to regurgitate from the intestine into the stomach. The test solution itself was prevented from regurgitating. In all seven of these experiments secretion of gastric juice was observed to commence within half an hour after the first appearance of bile in the stomach (table 1B). The volume of gastric secretion varied roughly in proportion to the volume of bile which regurgitated and the duration of contact of the bile with the gastric mucosa. Similar results were obtained in the other dogs but these were difficult to evaluate quantitatively because of the admixture of bile with the gastric juice. Solutions in volumes of 100 cc. of dog or ox bile were placed in the main stomach of the Pavlov pouch dog in eight experiments. In six instances the secretion from the pouch amounted, on the average, to 6.2 cc. during the first hour following bile administration while the volume for the control hour preceding bile administration was only 0.7 cc. These experiments confirm the early work of Sokolov (1904) who found that bile placed into an isolated stomach would stimulate secretion from a Pavlov pouch.

2. Presence of bile in the intestine. A free flow of bile regularly followed introduction into the intestine (or perfusion through the Thiry-Vella loop of dog L) of either 5 or 10 per cent proteose solution ("Bactoprotone") or 7.5 per cent amino acid mixture. The solutions were infused into the intestine in volumes of 100 to 300 cc. over a period of one to three hours. Thick dark bile usually

appeared within four to seven minutes. (In two experiments on dog M the infusion of "Bactoprotone" into the lower ileum was not followed by a flow of bile.) When the bile was excluded from the stomach and intestine by means of the suction apparatus an unequivocal secretion of acid gastric juice occurred in only five of twenty-five experiments, after an interval ranging between one and one-half and two and one-half hours. On the other hand, when dog bile was mixed with the proteose solution and infused into the intestine, gastric secretion appeared in seven of ten experiments and began after a latent period of one to

TABLE 1

Secretion from Pavlov pouch of dog B

	300 cc. of 10 per cent proteose solution infused into intestine for three hours and bile drained continuously from intestine							
	A Bile reflux into stomach prevented		B Bile refluxed into stomach during third hour			C Bile reflux into stomach prevented; 100 cc. dog bile infused into intestine during period of proteose infusion		
	Vol.	Mucus	Vol.	Total acid	Mucus	Vol.	Total acid	Mucus
				mgm. HCl			*mgm. HCl*	
Control period, cc. per 30 min.	1.8	–	2.0		++++	0.9		++
Infusion period, cc. per hour.								
1st hour	0.6	–	3.0		+	2.5		–
2nd hour	2.7	++++	3.8		–	12.0		+
3rd hour	4.3	+++	9.2		–	12.6		–
Total output	7.6		16.0	54.89		27.1	140.07	
Period after infusion, cc. per hour								
1st hour	2.6	+++	4.6		–	2.1		–
2nd hour	1.1	++	1.0		+++	–		–
3rd hour	1.4	++	–		–	–		–

two hours. Typical results of the two types of experiments are contrasted in the accompanying table (table 1, A and C).

Contrary to the results of Meyer, Ivy and McEnery (1924), we found that bile alone in the intestine did not stimulate gastric secretion. In fourteen experiments on fasting dogs from 50 to 200 cc. of either dog bile or 5 per cent ox bile was instilled into the intestine over periods of time ranging from seventy-five minutes to almost four hours. In only one experiment was a definite secretion obtained from the stomach. Apparently bile acts in some manner to make other stimuli effective.

3. *Osmotic activity of stimulating material.* An important factor in determining

the secretory response was found to be the concentration of the substance introduced into the small intestine. For example, infusion of isotonic or slightly hypertonic solutions of amino acids[3] in amounts up to 300 cc. was without any demonstrable secretory effect; on the other hand, more concentrated solutions (9.8 and 15 per cent) resulted in a gastric secretory response. In control experiments isotonic solutions of sodium chloride were without effect but solutions of sodium chloride isosmotic with the more hypertonic solutions of amino acids gave comparable secretory effects.

4. Volume of fluid introduced. The volume of fluid did not, per se, appear to be a factor in the excitation of the gastric glands. This was demonstrated both by experiments with isotonic saline and by those experiments with amino acids in which the response was negative. When the gastric glands were at rest, introduction into the samll intestine of as much as 760 cc. of isotonic sodium chloride solution over a period of two and one-half hours was without any secretory effect. However, as some of us have shown previously (Friedman, Pincus and Thomas, 1944), large volumes of fluid increase the secretory response to certain effective stimulating agents introduced subsequently into the intestine.

5. Effect of stumuli during "spontaneous" gastric secretion. While most of the experiments were carried out only when the stomach was in a state of secretory rest, on several occasions beef extracts or products of protein digestion (e.g., "Bactoprotone") were administered into the intestine at a time when the stomach showed secretory activity of unexplained origin. The effect of the intestinal stimulus on the "spontaneous" gastric secretion was not constant but in the majority of cases the rate of secretion appeared to be increased.

6. Distention of the intestine and vomiting. On several occasions a bout of retching and vomiting occurred during an experiment, at times apparently associated with too rapid inflow of fluid into the intestine. In two instances when vomiting occurred during a period of gastric secretion resulting from instillation of bile into the stomach, it was followed by brief augmentation of the rate of gastric secretion. This contrasted to the experience of Thomas and Crider, reported by Thomas (1943), who saw a marked decrease in volume and acidity of gastric secretion during nausea in animals secreting in response to normally ingested food. On the other hand, if retching or vomiting took place during a period when little or no secretion was in progress, we observed that secretion of gastric juice was initiated within ten to twenty minutes. The secretion was at first mostly mucus but was soon followed by acid. The volume and duration of the secretion varied roughly with the degree of apparent nausea exhibited, but in most instances lasted only thirty to forty-five minutes.

DISCUSSION. The evidence for the occurrence of the intestinal phase of gastric secretion is based largely upon experiments in which food or various products of digestion have been introduced into the intestine of fasting animals and secretion of gastric juice has been observed to follow. In our experiments, in which substances believed to be potent gastric secretory stimulants were introduced into

[3] The amino acids were supplied through the generosity of Dr. R. M. Johnson of Frederick Stearns & Co., Detroit.

the intestine of fasting animals with normally innervated stomach or gastric pouch, gastric secretion did not follow regularly except under special conditions. These were: presence of bile in the stomach or intestine; intestinal irritation due to hypertonic solutions; a pre-existing state of continuous gastric secretion; and, possibly, overdistention of the intestine. We know of no previous experiments in which all of these conditions have been controlled adequately. It appears, therefore, that the belief in the occurrence of an intestinal phase of gastric secretion directly due to the presence of food substances in the intestine is based on incomplete evidence.

Perhaps the greatest surprise, at least to us, was the failure of amino acids to stimulate gastric secretion when administered in isotonic or only slightly hypertonic solution. Concentrated solutions of amino acids were effective but so, too, were hypertonic solutions of sodium chloride. That concentration and not the total amount of amino acid administered was the determining factor was shown by two experiments, one in which a 7.5 per cent solution and another in which a 9.87 per cent solution of amino acids was instilled. With the more hypertonic solution secretion of gastric juice began after 9.5 grams had been infused, while with the other solution no secretion was obtained even though a total of 24.5 grams was infused. From the experiments of Ivy and others (Ivy, Lim and McCarthy, 1925) with hypertonic solutions of sodium chloride, it is probable that the secretory response to the concentrated solutions is associated with irritation of and damage to the intestinal mucosa. An additional factor may be more rapid absorption of amino acids from the concentrated solutions with consequent higher blood levels. Inasmuch as during the course of intestinal digestion following a meat meal the concentrations of amino acids in the intestine probably do not attain hypertonic levels, it is unlikely that these mechanisms are normally involved in stimulating gastric secretion.

Our experiments do not exclude the possibility that an "intestinal phase" may occur in the normal course of digestion since bile is usually present in the intestine along with products of protein hydrolysis. Rather, they serve to emphasize the importance of bile as a factor in the complex mechanism of the intestinal phase. Furthermore, the suggestive results obtained in continuously secreting animals may be explained on the assumption that individually inadequate intestinal stimuli may reinforce other stimuli in such a way as to increase materially the flow of gastric juice. Such a mechanism would be indicative of a useful adaptation, whereas an independent intestinal phase may be not only useless but possibly harmful.

SUMMARY 1. Isotonic solutions of proteose ("Bactoprotone"), amino acids or sodium chloride failed to elicit secretion of gastric juice in fasting dogs when administered into the intestine in the absence of bile. Bile alone was also without effect.

2. A moderate amount of gastric secretion was obtained after a latent period of from one to two hours when "Bactoprotone" mixed with bile or hypertonic solutions of amino acids or sodium chloride were placed in the intestine.

3. Previous observations that bile in the stomach causes secretion of gastric juice were confirmed.

CONCLUSION

Products of protein digestion alone do not elicit an intestinal phase of gastric secretion.

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SOME FACTORS AFFECTING THE RESISTANCE OF EJACULATED AND EPIDIDYMAL SPERMATOZOA OF THE BOAR TO DIFFERENT ENVIRONMENTAL CONDITIONS[1]

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Received for publication March 22, 1944

General observations in this laboratory have revealed that ejaculated spermatozoa from the boar cannot withstand adverse environmental conditions as well as epididymal spermatozoa. It appears that the decrease in resistance of spermatozoa as they pass from the reproductive tract into the semen may be associated with physiological and morphological changes within the spermatozoa. The possibility that accessory secretions may have a detrimental effect on the spermatozoa and bring about changes which lessen their ability to survive a cold shock and storage conditions also exists. On the other hand, epididymal secretions may increase the resistance of spermatozoa in a manner similar to that described for egg yolk diluters by Lasley, Easley and Bogart (1942), and Lasley and Mayer (1944) for bull spermatozoa.

Young (1929) found that the heat resistance of guinea-pig, rat and ram spermatozoa decreases as the spermatozoa move from the head to the tail of the epididymis. It is possible that the progressive decrease in resistance of spermatozoa might be a continuation of processes in spermatozoan development initiated in the testes and continued in the epididymis.

The results in this paper pertain to differences in the resistance and survival capacity of spermatozoa which may be associated either with changes in their environment or with changes within the cell occurring at different levels of the reproductive tract.

MATERIALS AND METHODS. The material for this study was secured from 20 boars ranging from 6 to 24 months of age. Methods used for obtaining both epididymal and ejaculated spermatozoa and for performing resistance and storage tests were described or references given to these methods in previous publications (Lasley and Bogart, 1943).

In the study of the influence of accessory secretions[2] on the resistance and survival of epididymal spermatozoa, centrifuged semen plasma was used for diluting the epididymal suspensions[3] by adding 2 parts of semen plasma to 1 part of the epididymal suspension. A control sample consisted of the non-diluted epididymal spermatozoan suspension as collected.

[1] Contribution from the Dept. of Animal Husbandry, Missouri Agricultural Experiment Station, Journal Series no. 944.

[2] Accessory secretions as used in this investigation consisted of secretions from the prostate, seminal vesicles, Cowper's and urethral glands plus the fluids coming from the epididymides and testes.

[3] Spermatozoa from the epididymis suspended in the fluids from the epididymis and the testis. In addition, some of the buffer used in collecting the spermatozoa make up part of the fluids.

The influence of epididymal secretions on ejaculated spermatozoa was studied by diluting the plasma-free ejaculated spermatozoa with sufficient centrifuged epididymal secretions to bring the volume back to its original level. All centrifuging was done by subjecting the fluids to a force of 1400 times gravity for 10 to 15 minutes.

RESULTS. *The influence of accessory secretions on the resistance and survival of epididymal spermatozoa.* The accessory secretions were not harmful to epididymal spermatozoa, but increased slightly the ability of these cells to withstand a cold shock and storage conditions (table 1).

TABLE 1

The influence of the accessory secretions on the resistance and survival of epididymal spermatozoa (average of 9 expts.)

TREATMENT	PERCENTAGE OF LIVE SPERM	
	Epididymal sperm + semen plasma	Epididymal sperm in suspension
As collected	88.3	88.3
0°C. for 10 minutes	56.9	54.0
6 days' storage	73.8	61.5
16 days' storage	46.7	25.0

TABLE 2

The influence of dilution on the resistance of epididymal spermatozoa in the boar

NUMBER OF BOAR	KIND OF DILUTER	PERCENTAGE OF LIVE SPERM (0°C. FOR 10 MIN.)					
		No dilution	1:2	1:4	1:8	1:16	1:32
81	Phosphate buffer*	68.0	71.0	72.0	73.0	74.0	73.0
34	Phosphate buffer*	84.0	86.0	87.0	83.0	85.0	85.0
13	Phosphate buffer*	59.0	59.0	59.0	59.0	58.0	59.0
101	0.9% NaCl	50.0	53.0	55.0	52.0	53.0	53.0
2	Semen plasma	80.0	78.0	82.0	77.0	82.0	78.0
Average...		68.2	69.4	71.0	68.8	70.4	69.6

* $\frac{M}{8}$ mono-potassium, di-sodium phosphate; pH = 7.4.

Since the concentration of the spermatozoa in the epididymis was usually between 4,000,000 and 5,000,000 per cu. mm. as compared to 400,000 to 500,000 per cu. mm. in the normal ejaculate, it is possible that the dilution of 2:1 in the experiments given in table 1 was insufficient to bring about any changes in the spermatozoa. To test this point, epididymal spermatozoa from 5 different boars were diluted in the ratios of 1:2, 1:4, 1:8, 1:16, and 1:32 and their resistance to a cold shock was determined. The results, summarized in table 2, indicated that dilution as high as 1:32 with several different substances had no significant influence on the resistance of the spermatozoa.

The influence of epididymal fluids on the resistance of ejaculated spermatozoa.

The greater resistance of epididymal spermatozoa to adverse conditions suggested the possibility that epididymal secretions might increase spermatozoan resistance. Four trials were made to test the action of epididymal secretions on the resistance of ejaculated spermatozoa. The results (table 3) show that in only 1 of 4 cases did the epididymal secretions influence the resistance of the ejaculated spermatozoa, and in this one the increase was slight and could have been due to experimental error.

Epididymal and ejaculated spermatozoa were diluted with egg yolk-phosphate buffer before a cold shock and the results (table 4) show that the resistance of both

TABLE 3

The influence of epididymal secretions on the resistance of ejaculated spermatozoa to cold shock

NUMBER OF BOAR	NORMAL SEMEN		EJACULATED SPERM + EPIDIDYMAL SOLUTION	
	As collected	0°C. for 10 minutes	As collected	0°C. for 10 minutes
54	91.0	6.0	92.0	6.0
81	64.0	5.0	64.0	5.0
13	86.0	14.0	87.0	24.0
2	90.0	5.0	89.0	4.0
Average.....	82.8	7.5	83.0	9.8

TABLE 4

The influence of egg yolk-buffer on the resistance of epididymal and ejaculated spermatozoa of the boar subjected to cold shock

(% live spermatozoa after 10 min. at 0°C.)

NUMBER OF BOAR	EPIDIDYMAL SPERM		EJACULATED SPERM	
	Non-diluted	Egg yolk-buffer	Non-diluted	Egg yolk-buffer
113	74.0	86.0	14.0	29.0
91	18.0	46.0	12.0	21.0
53	77.0	89.0	6.0	37.0
63	72.0	84.0	21.0	53.0
160	87.0	88.0	12.0	20.0
Average.....	65.6	78.6	13.0	32.0

ejaculated and epididymal spermatozoa was increased by the addition of egg yolk-phosphate buffer.

The relation of resistance and storage to physiological development of spermatozoa. The foregoing data have shown that the accessory secretions and epididymal fluids had little influence on the resistance of boar spermatozoa. However, one other factor, the physiological development of the spermatozoa, may be of importance. In order to test this factor, samples of spermatozoa were taken from different levels of the reproductive tract and subjected to a cold shock. A portion of each sample was also stored for periods of 6 and 16 days to determine the ability of the spermatozoa to survive the different storage periods. The results (table 5) show that the spermatozoa become progressively less resistant to a cold

shock and to storage conditions as they move along the reproductive tract through the epididymis and vas deferens.

Additional data (table 6) demonstrate the marked lowering in the resistance of spermatozoa as they pass through the reproductive tract and are ejaculated as a component of semen. A striking phenomenon demonstrated by these data is the sharp decrease in resistance of spermatozoa upon leaving the vas deferens and becoming a part of the ejaculate.

DISCUSSION. A number of workers hold the opinion that the accessory fluids shorten the life of spermatozoa stored "in vitro". Since spermatozoa at ejaculation are diluted as much as 10 to 15 times with the secretions of the accessories, it seemed possible that these fluids might be responsible for the lowered resistance of spermatozoa in semen. Gunn (1936) reported that ram ejacula containing a

TABLE 5

The storage potentialities and resistance of spermatozoa from different parts of the reproductive tract (average of 3 samples)

TREATMENT	PERCENTAGE OF LIVE SPERMATOZOA		
	Body of epididymis	Tail of epididymis	Vas deferens
As collected	94.0	92.4	92.1
Cold shock (0°C. for 10 minutes)	62.5	55.8	48.0
Stored 6 days	87.1	82.0	76.4
Stored 16 days	47.9	38.4	34.2

TABLE 6

The resistance of boar spermatozoa in the different regions of the reproductive tract and in normally ejaculated semen

NUMBER OF BOAR	PERCENTAGE OF LIVE SPERMATOZOA (0°C. FOR 10 MIN.)			
	Body of epididymis	Tail of epididymis	Vas deferens	Normal semen
81	82.0	80.0	70.0	5.0
13	68.0	59.0	51.0	14.0
63	72.0	70.0	34.0	21.0

large proportion of accessory fluids had a comparatively short life, and he attributed this to the rapid expenditure of energy in the presence of the accessory fluids. Milowanov and Selivanow (cited by Bernstein and Sokolowa, 1935) suggested that the secretions of the accessory glands injured boar spermatozoa by destroying the protective lipoid capsule which supposedly surrounds each spermatozoon. No harmful effect of the accessory fluids on the resistance of spermatozoa from the epididymis of the boar to a cold shock or survival under storage conditions was observed in this study. On the contrary, the resistance and storage potentialities of spermatozoa apparently increased in the presence of the accessory fluids.

The dilution of suspensions of epididymal spermatozoa as much as 1:32 with various substances did not lower the resistance of these cells. Young (1929)

found that the heat resistance of guinea-pig spermatozoa was decreased considerably when diluted excessively with Locke's solution. However, Young's conclusions were based on spermatozoan motility. It is possible that the duration of motility might decrease without death of the spermatozoa. In Young's experiments, the spermatozoan suspensions were placed in a water bath at a temperature of 46°C., and the time required to stop the motion of all spermatozoa was recorded. Therefore, the samples of greater dilution necessarily contained fewer spermatozoa which, on a motility basis, gave an apparent decrease in resistance. Our experiments differed from Young's in that the resistance of the spermatozoa in each sample was based on the actual per cent of live cells in a count of 500 spermatozoa on each of two slides.

The influence of the epididymal secretions on the resistance of ejaculated spermatozoa was studied because of the reported protective action of the epididymal secretions. According to Redenz (see Walton, 1933) during their residence in the epididymis the spermatozoa receive a coating of colloidal material from the walls of the epididymis which renders them more resistant to electrolytes. Gunn (1936) accepts this theory and considers the increased longevity of spermatozoa from rams previously injected with pituitary emulsions to be due to a strengthening effect of the epididymal secretions which were stimulated by the testis hormone.

Spermatozoa in ejaculated boar semen have practically no resistance to a cold shock and their survival during storage is very low. Placing epididymal spermatozoa under similar environment to that of ejaculated spermatozoa by the addition of semen plasma to the former does not lower their resistance or ability to survive during storage. Also, the secretions in which epididymal spermatozoa exist do not alter the ability of ejaculated boar spermatozoa to withstand a cold shock or to survive a long storage period. If, on the other hand, ejaculated or epididymal spermatozoa are diluted with egg yolk-phosphate buffer, their resistance to a cold shock and their ability to survive during storage are increased This suggests that boar spermatozoa also have the ability of becoming resistant in the presence of egg yolk. This is comparable to the behavior of bull spermatozoa in egg yolk diluter as shown by Lasley, Easley and Bogart (1942), Easley, Mayer and Bogart (1942), Lasley and Bogart (1943), and Lasley and Mayer (1944). A resistance factor evidently is not present in the fluids of the epididymis, because diluting ejaculated spermatozoa with these fluids did not increase their resistance even though they were capable of becoming resistant in egg yolk-phosphate buffer. It, therefore, seems logical to conclude that the ability of epididymal spermatozoa to resist adverse conditions is determined by the cells rather than the environment in which they exist. The resistance of spermatozoa which progressively decreases as they move from their place of origin to the point where they become a component of semen, then, is influenced by changes within the cell rather than by changes in the environment. It is possible, however, that the environment to which spermatozoa are subjected may alter the rate of change which occurs within the cells.

The results reported in table 4 show that the resistance of ejaculated boar spermatozoa to a cold shock may be increased by the addition of egg yolk-phosphate buffer. However, the resistance of these ejaculated spermatozoa diluted

with egg yolk-phosphate buffer did not equal that of undiluted spermatozoa from the epididymis. Furthermore, the increase in resistance from 13 to 32 per cent was not as great as that reported by Lasley and Bogart (1943) or Lasley and Mayer (1944) for bull semen. The resistance of spermatozoa from bulls was increased from 13.1 per cent in non-diluted semen to 56.4 per cent in semen diluted with egg yolk-phosphate buffer. All the evidence in these studies suggests that the cause of the reduced resistance and lowered ability of ejaculated spermatozoa to store resides in the cell itself rather than in the secretions within which it exists, and this contention is confirmed by the results of Lasley and Mayer (1944) who investigated the comparative resistance of ejaculated and epididymal spermatozoa of the bull.

CONCLUSIONS

1. Secretions from the accessory glands did not greatly influence the resistance to a cold shock or storage potentialities of boar epididymal spermatozoa.
2. The degree of dilution, using several different diluters, had no influence upon the resistance of epididymal spermatozoa to a cold shock.
3. The fluids from the epididymis, obtained by centrifuging boar epididymal suspension, did not influence the resistance to a cold shock or storage potentialities of ejaculated boar spermatozoa.
4. Diluting suspensions of epididymal and ejaculated boar spermatozoa with egg yolk-phosphate buffer increased their resistance to a cold shock and their survival under storage conditions.
5. The resistance of spermatozoa to a cold shock and their ability to survive during storage varies with the place in the reproductive tract from which they are obtained. Spermatozoa from the head of the epididymis are very resistant and survive during storage for long periods but their resistance and survival capacity decrease as the distance of their location from the testis increases until spermatozoa in the ejaculate have practically no resistance or storage potentialities.
6. It is suggested on the basis of the foregoing observations that the reduction in resistance and storage potentialities of boar spermatozoa are associated with changes within the spermatozoa rather than with changes in their environment.

Acknowledgment. Glynden T. Easley assisted with castrations and some of the laboratory studies, and Professors E. A. Trowbridge and Dennis T. Mayer helped in the preparation of this manuscript.

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COMPARISON OF DIRECT AND INDIRECT BLOOD PRESSURE MEASUREMENTS IN RATS

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Received for publication April 3, 1944

It has been shown that the results of the indirect (cuff) method of determining blood pressure in man must be standardized before they may be accepted as accurate measurements of the arterial pressure (1, 2, 3, 4, 5). Using the ordinary clinical procedure controlled by simultaneous measurement with an optical manometer of adequate frequency inserted directly into the artery it has been found that the cuff readings will not measure the true arterial pressure unless the width of the cuff is correct in relation to the circumference of the arm.

It is common practice to determine the blood pressure of the rat by the method of Williams, Grollman and Harrison (6). This method involves the same general principle as the ordinary clinical method used on man. Figures got by this and similar methods (7, 8) are much lower than those obtained in the rat by intra-arterial manometry (9). Moreover, these figures indicate the incredible notion that the systolic pressure is of the same order as (or lower than) the mean pressure measured by arterial cannulation and the mercury manometer (10).

These contradictions led us to investigate the rat method and to check it against an optical manometer. A clue as to what might be wrong is seen in the fact that whereas the best cuff width to measure the blood pressure of the human infant is one inch (2 cm.) it is common practice to use a cuff 4 cm. (6) or 26 mm. (7) wide on the rat's tail. These cuffs might be thought to be too wide and to give readings that are too low when the comparative sizes of the rat's tail and the infant's arm are kept in mind.

APPARATUS. The optical manometer used was of the the hypodermic type (1, 11) equipped with a blunted 26 G. Luer needle, a short leaden tube and a heat treated beryllium copper membrane 0.0009 inch thick formed into a "pie plate" shape to insure proportionality between pressure and deflection and to insure a stable zero. The natural frequency of the manometer as used is about 200 cps. A typical record is shown in figure 1. Systolic pressure was measured by means of photographed calibrations at the peak of the pulse curves. The pressure pulses could be measured to within 2 or 3 mm. Hg.

For some time it has been the practice in this laboratory to insert a stopcock between the manometer and the leaden tube to facilitate calibration, to fix a loop in the leaden tube for distributing bending strain and to have the stopcock which is placed between the manometer and pressure on a separate fitting that will screw into the manometer. A very slow stream of saline can enter the manometer system through this stopcock serving to prevent clotting up the needles over long periods of time and to minimize deterioration of the animal (dog). Pressure registration is in no way interfered with.

The apparatus for indirect measurement of blood pressure was made on the plan of Williams, Grollman and Harrison and a number of fairly convincing estimations of blood pressure were made. It was not easy to be sure of the end point because, even though the animal was warmed according to directions, the fluid moved up in the oncometer tube such a small distance that much argument ensued as to whether there was an end point, and if so at what pressure.

To make the tail plethysmograph a more sensitive indicator of the expected increase in volume when the cuff allowed blood to fill up the tail vessels the oncometer was lined with condom rubber tubing instead of cigarette drain tubing and its distal end was plugged and sealed. There was definite improvement in the way the apparatus worked but we were still occasionally at odds as to our end points. This led us to substitute a very narrow ($\frac{1}{2}$ mm.) tube for the $\frac{1}{8}$ inch water tube which had been used to indicate the volume increase of the tail. The end point was much sharper and more reproducible with the capillary tube. The water level moved up in the oncometer tube 4 to 10 mm. sharply and quickly as the cuff pressure fell. This is to be expected because we are probably trying to observe the effect of the entrance into the system of 1 to 5 cmm. of blood.

Fig. 1. Pressure pulse from the carotid artery of the rat

The advantage of the smaller tube becomes obvious when we figure that it multiplies the movement by 36. The effect of capillarity can be ignored, since the pressure in the chamber around the tail is set at an optimum which is determined by experience and can be varied at the discretion of the operator.

It was also found that when the capillary tube was used it was not necessary to warm the rat or train the observer. We found no useful difference in end point sharpness whether the rats were warmed or not as long as the small tube was used, but we did find warming necessary when using the large tube. The elimination of the complicated apparatus and procedures involved in warming the rat has obvious advantages, nor is it self-evident that the blood pressure level is itself unaffected by warming the animal.

To investigate the effect of cuff width on the relation between true pressure and cuff readings six interchangeable cuffs were made. They were like those described in the literature (6, 7) except that a removable collar was designed to fit between the cuff and the oncometer. The inside lengths of six cuffs were 36, 26, 16, 7, 5 and 3 mm.

Procedure. To compare the direct and indirect methods of measuring blood pressure, experiments were performed on six rats anesthetized with nembutal.

The anesthetized rat was placed in the rat holder and its tail passed through a selected cuff and into the oncometer. A carotid (usually the left) was cannulated with the manometer needle. Blood was forced out of the tail veins by raising the pressure in the oncometer to 25–30 cm. H_2O. The cuff pressure was raised to 200 mm. Hg and the oncometer pressure lowered to about 5 cm. H_2O. The photokymograph was then started and the cuff pressure lowered slowly. When the blood entered the tail and forced up the level in the water tube a signal was made on the record. The cuff pressure at this moment was compared with the recorded systolic pressure. In some experiments a second optical manometer was used to indicate pressure in the cuff, thus eliminating any possible error in reading the mercury manometer. Measurements showed that such error was negligible. Comparisons were made when possible between the directly recorded pressure and readings taken from all cuffs on each rat. A total of 168 comparisons were made at various levels of blood pressure. Many cuff readings were taken on conscious, intact rats as well and their interrelationships found to be consistent with those described below.

Results. The results of comparing true systolic pressures with cuff systolic pressures are shown in figure 2. The 3 mm. cuff did not stop blood flow unless it was blown up to 270 mm. Hg and the use of this cuff was discontinued after a few trials.

The 5 mm. cuff gave results that were on the average closest in agreement with the true pressures. At high pressures the cuff readings were too high and at low pressures they were too low.

The 7 mm. cuff gave variable results that were on the average low.

The 16 mm. cuff gave readings 36.5 ± 5.6 mm. Hg too low. They tend to make a line that is parallel to the line of agreement between true pressure and cuff readings and there is no tendency to average either at high or low pressures.

The 26 and 36 mm. cuffs give readings that are low and distinctly more variable than those made with the 16 mm. cuff.

Discussion. The 5 mm. cuff gave readings which are in best agreement with the true systolic pressures. The comparisons group themselves along a line which intersects the line of best agreement at 120 mm. Hg. The cuff gives falsely low readings at low pressures and falsely high readings at high pressures. To correct the 5 mm. cuff readings one could add roughly 7 mm. Hg when the reading is 100, 14 mm. when the reading is 80 and one could subtract 7 and 14 mm. when the readings exceed 120 by 20 and 40 mm. Hg. Whether this procedure could be extrapolated into truly hypertensive ranges or not cannot be said at present.

If one should decide to use the 16 mm. cuff he could correct his readings to the true systolic pressure by adding 36.5 ± 5.6 mm. Hg to the readings. There does not seem to be any tendency for low and high readings to show different errors.

To salvage the measurements which have appeared in the literature and which were made with 26 and 40 mm. cuffs, our data indicate that they could be made more or less congruent with reality by adding 40 ± 10.7 mm. Hg. The random error with the wide cuffs is greater than with the narrower cuffs. The 40 mm. gap between these figures and true systolic pressures should not be ignored.

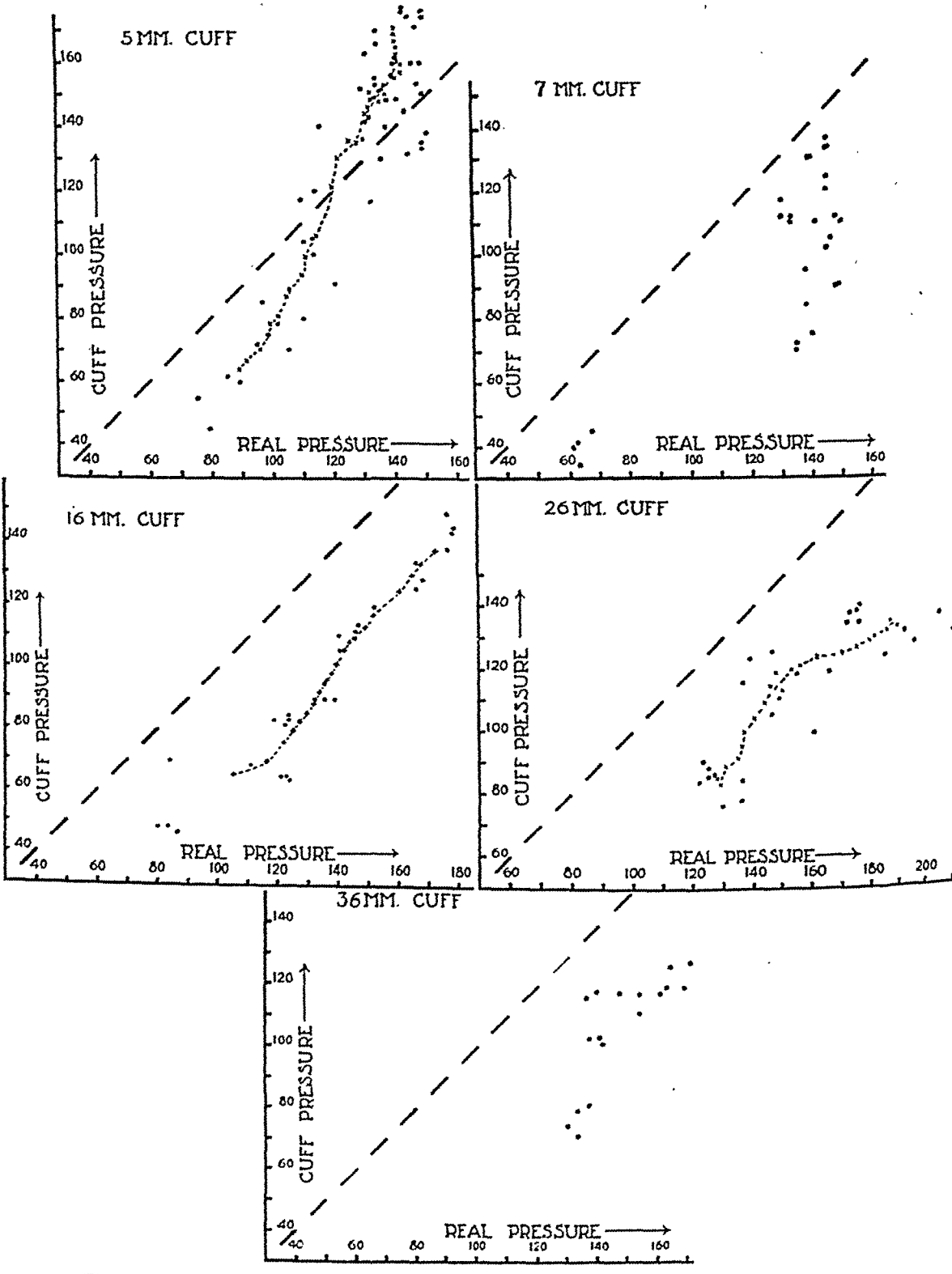

Fig. 2. Plot of cuff readings against hypodermic manometer readings. x--------x--------x represents figures obtained by averaging successive sevens. The diagonal line represents perfect agreement.

Systolic pressure in the tail arteries probably is no higher than that in the carotid because the arterial tree is so small and quick-acting that it cannot be thrown into free oscillations (standing waves) as can the arterial tree of dogs and men (1, 9, 12). Resistance to flow in the large arteries of the abdomen and tail is such that the diastolic and mean pressures in the rat's tail arteries are probably only slightly less than in the carotid.

SUMMARY

1. Simultaneous readings of systolic pressure of rats from the Williams, Harrison, Grollman (6) apparatus and from direct arterial puncture showed that pressures obtained by them were too low and that the disagreement was variable. Addition of 40 ± 10.7 mm. Hg to published figures will bring them to about the right value.

2. Substitution of a 16 mm. cuff for the 40 mm. cuff (6) or 26 mm. cuff (7) gave readings which were consistently 36.5 ± 5.6 mm. Hg low, and which were less variable.

3. A 5 mm. cuff gave much truer readings. They were accurate at 120 mm. Hg, but somewhat too high above that level, and too low below that level. These errors can be corrected (see text).

4. The use of a ½ mm. capillary tube for the oncometer gave sharper end points, greater reproducibility and eliminated the need of heating the rats and of training the observer.

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THE LACTATE RESPONSE TO EXERCISE AND ITS RELATIONSHIP TO PHYSICAL FITNESS

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Received for publication April 8, 1944

Since the demonstration by Ryffel (1909) of an increased lactic acid concentration in the blood and urine of man after muscular activity, a number of reports have appeared on the general subject of lactic acid in relation to exercise. From the communications of Hill, Long and Lupton (1924); Liljestrand and Wilson (1924); Jervell (1928); Margaria, Edwards and Dill (1933); Dill, Edwards, Newman and Margaria (1936); Bang (1936); Newman, Dill, Edwards and Webster (1937) and Johnson and Edwards (1937), it is now possible to present a picture of the changes in blood and urine lactate during and following muscular activity. With the onset of exercise there is a rapid increase in the blood lactate concentration to a maximum; the magnitude of its maximum is greater, other factors remaining constant, the greater the intensity of exercise (Bang, 1936). The view has been expressed that this maximum concentration, once attained, remains constant during a continued steady state exercise (Hill, 1926). A review of the literature reveals little evidence to support this and Bang (1936) has actually demonstrated that the blood lactate in man decreases even though the activity is continued in a steady state. Confirmation of this has come with the report of Flock, Ingle and Bollman (1939) that in the stimulated muscles of the rat the lactate content rises rapidly during the first minute and then declines, this decline being similar whether the muscle continues to be active or not following the initial stimulation.

With cessation of exercise the blood lactate decreases, the recovery rate being such that the logarithm of the excess lactate is a linear function of time (Margaria, Edwards and Dill, 1933; Dill, Edwards, Newman and Margaria, 1936). Under some circumstances, especially with exercise of short duration, the blood lactate concentration continues to rise for some minutes after cessation of the activity (Laug, 1934; Dill, Edwards, Newman and Margaria, 1936; Bang, 1936). Investigators differ as to whether this rise is due to a lag in the diffusion of lactate from the muscles into the blood (Bang, 1936) or to an actual post-exercise production of lactic acid (Margaria, Edwards and Dill, 1933; Newman, 1938).

Following exercise of sufficient intensity, lactate appears in the urine. Liljestrand and Wilson (1925) noted quantities of this substance ranging from 140

[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Stanford University.

The authors wish to express their indebtedness to Dr. John Field II for the use of equipment and to Dr. F. W. Weymouth for advice on the statistical treatment of data. Acknowledgment is made of technical assistance by Ellen Burton, Royce Skow, Bernard Hansen and Robert Morris.

to 1370 mgm. over the resting level after a period of activity. The peak of lactate excretion occurs 10 to 20 minutes after the cessation of exercise (Jervell, 1928; Johnson and Edwards, 1937) and excretion is complete in 40 to 50 minutes after the cessation.

One of the many factors which may determine the lactate response of an individual to a given exercise is the individual's exercise tolerance (physical fitness). Several reports allude to the higher blood lactate following a submaximal exercise in persons considered unfit or untrained (Bock, Vancaulaert, Dill, Folling and Hurxthal, 1928; Owles, 1930) or to a lower critical intensity of exercise for unfit subjects beyond which increases in blood lactate appear (Cook and Hurst, 1933; Newman, Dill, Edwards and Webster, 1937). Bang (1936) cites the lower blood lactate response to exercise of two subjects after a two month training period, an observation which is confirmed by Edwards, Brouha and Johnson (1940). The lower blood lactate response to a submaximal exercise after a training program was also observed by Robinson and Harmon (1941), who also reported a higher blood lactate response to exhausting exercise after a training period: this latter finding has been confirmed by Knehr, Dill and Neufeld (1942). The urinary output of lactate in exercise also appears to be decreased after a training period, according to the results of Lewis, Hewlett and Barnett (1925). The modification of the lactate response by a training program is considered reliable enough by Johnson and Brouha (1942) to include it in an index of physical fitness.

On theoretical grounds there seems to be a real basis for a relationship between fitness and the lactate response. On the assumption that the lactic acid of exercise arises mainly as the result of anaerobic glycolysis (Bang, 1936; Jervell, 1928) in the muscles during the initial period before the circulatory and respiratory adjustments are completed, it is understandable how physical training, by making these adjustments more efficient either in speed or degree (or both) and thereby decreasing either the degree or duration of anaerobiosis in the muscles, can lower the lactate production in relation to a specific exercise. Since the results thus far are suggestive rather than statistically conclusive, it remains to be seen whether such a theoretical expectation is capable of experimental verification.

It is the aim of this report to present data on the blood and urine lactate response to exercise of a group of young men in several states of fitness; to illustrate the time course of the response in these individuals; and to examine the data for a possible quantitative relationship between fitness and some function of the lactate response.

PROCEDURE. The 47 subjects in these experiments were college students and laboratory workers, varying in age from 19 to 33 years. All were in good health and in respect to physical training ranged from men who take vigorous exercise regularly to laboratory workers who lead sedentary lives except for the activity involved in routine laboratory work. The lactate response of these individuals was studied in three types of muscular exercise:

I. Submaximal treadmill walk for 15 minutes. Speed was 100 meters per minute and the grade was 15 per cent.

II. Submaximal arm-and-leg stool stepping for 2 minutes with a step height of 18.5 inches and an ascent-descent rate of 40 per minute. In this exercise one hand grasped a horizontal bar 70 inches above the floor while the ipsilateral foot rested on the stool. The body was raised through a distance equal to the height of the stool by the simultaneous pull of the arm (flexion) and extension of the leg. At each cycle pull-up arm and step-up leg were changed thus giving to all the appendages an equal opportunity to share in the work. A knapsack for weights was carried on the back of the subject.

III. Treadmill exercise in three sections: (a) preliminary treadmill walk at 106 meters per minute and a 5 per cent grade which lasted for 4 minutes, (b) a sitting rest period of 4 minutes, (c) a treadmill run to exhaustion at 162 meters per minute starting at a 5 per cent grade and increasing the grade 1 per cent each minute until the subject quit.

Between 0.1 to 0.5 ml. of blood was collected in tubes coated with oxalate and fluoride, the blood being obtained from two closely spaced stab wounds in the finger tip. In the case of the treadmill walk (I) blood was collected before, during and for 60 to 90 minutes after cessation of the exercise. For the stool stepping exercise (II) blood was collected before and for 60 to 90 minutes after the end of the work. With the treadmill exercise (III) blood was obtained 2 minutes after the end of the preliminary walk (IIIa); 5 minutes after the end of the exhaustive run (IIIc) and 20 minutes after the end of the exhaustive run. In 12 subjects blood was also collected before the beginning of the walk (IIIa) and in two subjects blood was also obtained during the exhaustive run (IIIc).

In the case of types I and II tests, urine samples were collected before the beginning of the exercise and at intervals following the end of the exercise. Urines from subjects in the basal condition were collected after a 30 to 40 minute rest which was preceded by voiding all previously formed urine and imbibing 500 ml. of water.

The heart beat was recorded by means of the cardiotachometer of Henry (1937). The respiratory metabolism was also determined; for this purpose the air expired through either a face mask or mouth piece was collected in a 400 liter double chamber gasometer. Collections of expired air were made during the 9 to 14 minutes of the 15 minute treadmill walk (type I); during the 1.5 to 4 minutes of the preliminary treadmill walk (IIIa); and during the last minute of the maximal treadmill run (IIIc). Duplicate samples of this expired air were analyzed for oxygen and carbon dioxide by means of the Haldane apparatus. The blood and urine lactate concentrations were determined by the procedure of Barker and Summerson (1941). To indicate the standard error of these determinations, the results of duplicate analyses of blood collected from 31 subjects during the type III test have been treated statistically. This analysis covers a range of blood lactate from 20 to 300 mgm. per cent. In table 1 A are shown the mean blood lactate concentrations, the standard error (S.E.) and the coefficient of variability. The standard error is given by $\bar{D}/1.1284$, where $\bar{D}$ is the average difference between duplicate samples without regard to sign.

Results. I. *Resting blood and urine lactate.* The individual and mean values of the blood and urine lactate concentration of 11 subjects in the post-

absorptive, resting state are indicated in table 1 B. All the earlier literature (Jervell, 1928) gives somewhat higher values for resting blood lactate than are

TABLE 1 A

Duplicate analysis of blood for concentration of lactate during treadmill experiments (type III)

	MEAN	S.E.	C.V., S.D./MN.
	mgm.%	*mgm.%*	
Rest (not basal)	28.5	4.4	15.4
2 minutes after walk	32.5	2.8	8.6
5 minutes after run	215.9	10.3	4.8
20 minutes after run	143.1	10.9	7.6

TABLE 1 B

Post-absorptive, resting blood and urine lactates

SUBJECT	BLOOD	URINE LACTATE		
		Conc.	Total	Rate
	mgm.%	*mgm.%*	*mgm.*	*mgm./min.*
P. L.	7.3	2.12	0.70	0.016
	10.3	1.25	1.06	0.023
	14.5	1.11	1.33	0.024
R. S.	7.0	3.29	2.20	0.059
	11.3			
	13.4	0.76	1.19	0.028
	9.8			
	13.4			
	11.2	1.61	1.21	0.023
	14.8	1.47	1.31	0.034
	11.9	1.36	0.90	0.023
	8.4	2.93	2.43	0.053
	16.0	1.13	2.03	0.040
A. O.	9.4	0.49	0.96	0.022
	13.4	0.78	0.83	0.036
E. S.	14.3	1.60	1.20	0.028
B. H.	13.2	0.37	1.56	0.020
	11.3	1.82	0.88	0.021
	11.2	1.72	3.44	0.063
D. K.	15.1	0.60	2.54	0.073
T. B.	14.4			
R. U.	9.9			
C. T.	22.7	1.43	3.93	0.063
C. K.	13.2	2.05	1.46	0.042
Means	12.4	1.47	1.64	0.036

given in this table but a number of the more recent communications present values in about the same range. The results of Decker and Rosenbaum (1942), obtained also with the Barker-Summerson method, are somewhat lower than

the figures for blood lactate given here. The figures for resting urine lactate shown in table 1 B agree well with the few data available in the literature. Liljestrand and Wilson (1925) report an excretion of 0.1 to 0.25 mgm. lactate per minute while values of 0.013 and 0.015 mgm. per minute for two experiments are given by Johnson and Edwards (1937).

II. *Lactate response to submaximal exercise.* Nineteen subjects performed the 15 minute walk (type I). In all experiments each subject rested for at least 30 minutes on a bed before exercising, during which time samples of finger blood and all the accumulated urine were collected. Representative curves to indicate the time course of the blood and urine lactate changes in four subjects are presented in figures 1 and 2. Subjects B. H., F. C. and C. K. were in poor physical condition in contrast to J. D. who was a cross-country runner and was in excellent condition at the time of the test. These curves, along with the mean curves for all the subjects of figure 3, show that the blood lactate concentration rises rapidly after the beginning of exercise, reaches a peak, and then declines along a curve in which the rate of decrease is a function of the time after cessation of the exercise. Only 2 of the 20 subjects showed a higher blood lactate concentration within two minutes after the cessation of activity than the peak value during the walk. There is no suggestion in such a steady state exercise of a post-exercise rise in blood lactate concentration; neither is there any indication, within the limits of the time intervals involved, of the attainment of a steady state blood lactate concentration.

Identical experiments performed on the same individual on different days yield lactate response curves that indicate considerable day-to-day variability. This is seen in the duplicate test of F. C. (fig. 1) and the triplicate test of B. H. (fig. 2). A similar variability was obtained in the stool-stepping exercise (type II) where a much larger proportion of the body musculature is active than in the treadmill walk. These wide differences obtained on separate occasions, associated with no known change in the fitness of the subject, indicate the difficulty involved in obtaining a reliable figure based on only one or a few tests which is significant in characterizing the individual.

The time course of lactate elimination through the urine is also indicated in figures 1 and 2 where the rate in mgm./minute is plotted in each case versus the midpoint of the collection period, the duration of the collection interval, in minutes, being indicated by a printed figure adjacent to each point on the graphs. The amount of excess lactate that appears in the urine as a result of the exercise varies from individuals who show no increase (J. D.) to those whose urines excrete several hundred milligrams (B. H.). The major portion of the excess lactate is contained in the urine collected over the 10 to 20 minute interval after the end of the exercise. By the 40 to 50 minute period the urinary output of lactate has returned to the resting level. This time course of lactate excretion is similar to that already reported by Liljestrand and Wilson (1925) and Johnson and Edwards (1937).

III. *Lactate response to maximal exercise.* The blood lactate responses during and after the maximal treadmill run (type IIIc) are given for 2 subjects in figure

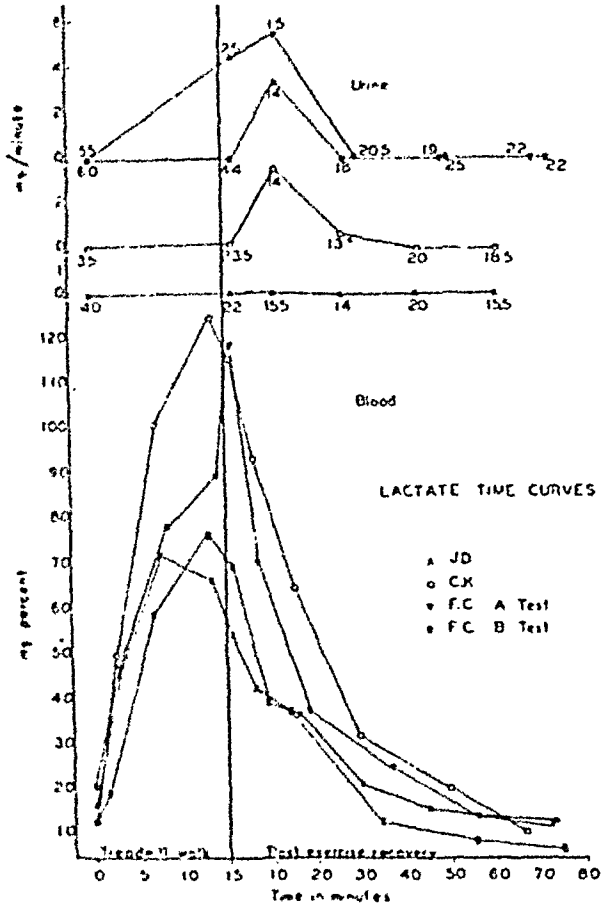

Fig. 1

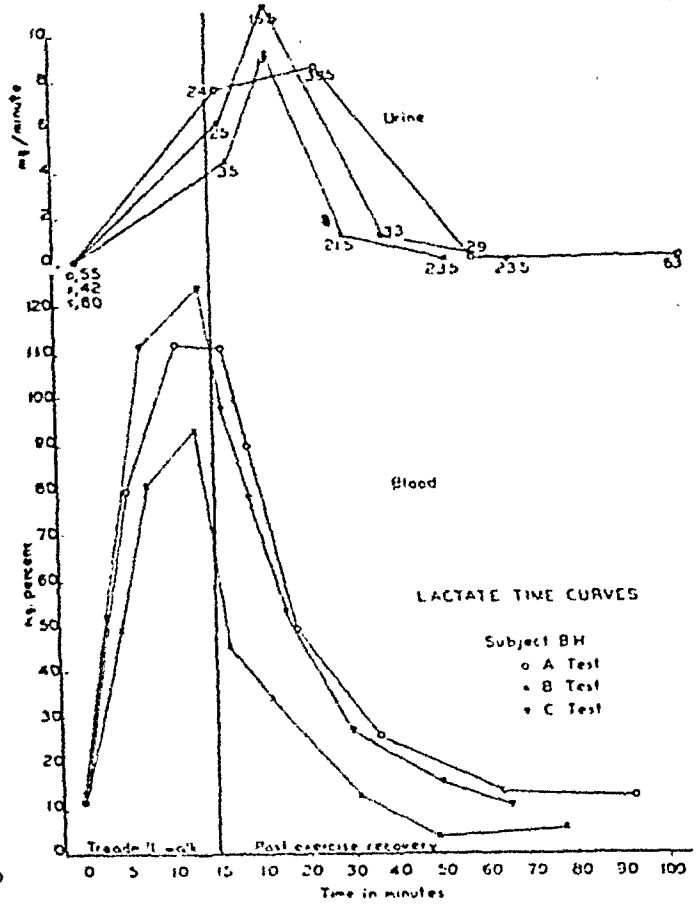

Fig. 2

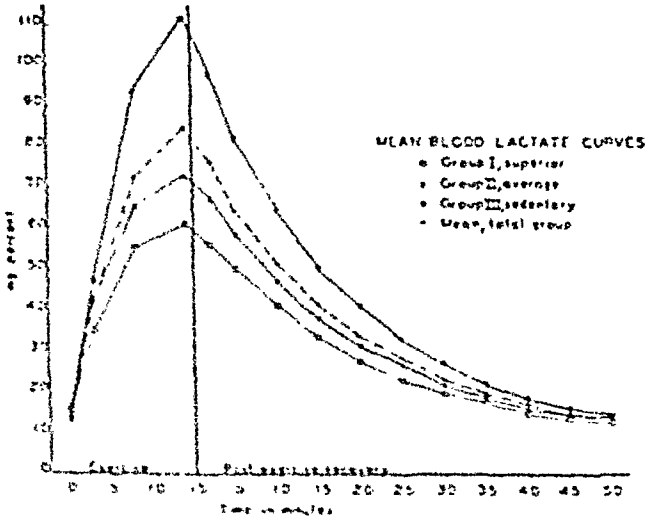

Fig. 3

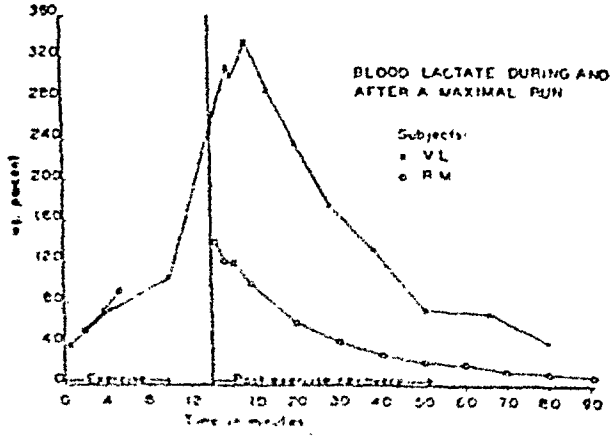

Fig. 4

4. Subject V. L. was an athlete who ran for the superior time of 13 minutes on the test while R. M. was a laboratory worker who continued for the slightly

below average time of 7 minutes. In the case of V. L. there is evidence of a post-exercise increase in blood lactate concentration for some minutes following the termination of the run. For the 31 subjects who performed the type IIIc exercise the mean value of blood lactate 5 minutes after the end of the run was 217.6 mgm. per cent with a range of 117 to 328 mgm. per cent; 10 minutes after the run, the mean value was 144.1 mgm. per cent with a range of 54 to 243 mgm. per cent. This wide scatter gives no evidence of a "ceiling" lactate level toward which the subjects approach when reaching the point of exhaustion.

IV. *Lactate response and physical fitness.* In order to elucidate the relationship between the lactate response and fitness an analysis has been made of the data obtained on the 19 subjects who performed the 15 minute treadmill walk (type I). These subjects have been segregated into the following 3 groups on the basis of personal knowledge of the subject's exercise background and on the basis of results obtained from performance tests used in this laboratory which are to be reported elsewhere.

Group I. Athletes or men who have performed in a superior manner in the laboratory.

Group II. Men who have taken regular but not competitive exercise.

Group III. Laboratory workers who exercise little or not at all.

The time course of blood lactate concentration for these three groups along with the mean curve for all 19 subjects is reproduced in figure 3. It is obvious that for both the exercise and post-exercise periods group I exhibits the lowest lactate level; group III, the highest lactate level; while group II occupies an intermediate position. To characterize these three groups more completely the results of other physiological measures have been combined with the blood lactate concentration at two points in the post-exercise period to yield table 2 A. From these data it appears that in contrast to an unfit subject, a fit man has, on the average, a lower post-exercise blood lactate concentration, a lower total excess urine lactate, a lower heart rate during the exercise, a higher oxygen consumption, a lower R.Q., and a lower ventilation. Using Fisher's t (Croxton and Cowden, 1942, p. 330) the data have been analyzed for significance of differences. The resulting t and P_t values for the comparison among the three groups, which are reproduced in table 2 B, are of interest in that they indicate that the differences between the means of the three groups do not attain statistical significance except for the extreme groups I and III. The oxygen consumption has no significance even for these extreme groups. It is notable that blood lactate and urine lactate may serve to differentiate fitness groups better than do heart rate and respiratory measures.

Reference has already been made to the variation in blood lactate response of the same individual when tested on several different occasions. This, of course, brings into question the reliability of any one lactate determination. This reliability has been assessed by computing the reliability coefficient (r; between test and retest blood lactate determinations) for the 31 subjects in the case of both the preliminary treadmill walk (types IIIa) and the maximal treadmill run (type IIIc). A value of unity for this coefficient would, of course, indi-

cate complete agreement between the test and retest determinations. For the preliminary walk the reliability coefficient was found to be 0.77; for the maximal run they were 0.71 and 0.81, respectively, for the 5 minute and 20 minute post-exercise values. These coefficients, though not high, compare favorably with the coefficients of other physiological measures which have been determined in this laboratory and are to be reported later. The conclusion seems warranted that for testing purposes, blood lactate determinations have at least as high, if not a higher, reliability than such measures as heart rate, ventilation, etc.

TABLE 2

Comparison of three fitness groups in 15 minute treadmill walk

A. Group means

	I	II	III
Blood lactate			
5 min. post-exercise	50.1	64.6	93.5
20 min. post-exercise	41.0	52.1	75.4
Excess urine lactate	4.33	18.8	165.5
Maximal exercise heart rate	166.2	172.4	180.5
Oxygen consumption	37.1	36.1	35.0
Carbon dioxide production	34.9	34.1	34.3
Respiratory quotient	0.94	0.94	0.98
Total ventilation	738	728	849

B. Significance of difference between groups

	I-II		II-III		I-III	
	t	P_t	t	P_t	t	P_t
Blood lactate						
5 min. post-exercise	1.27	0.50	2.16	0.06	3.78	0.003
20 min. post-exercise	1.20	0.26	1.91	0.09	3.23	0.009
Excess urine lactate	1.69	0.12	2.29	0.05	2.81	0.02
Maximal exercise heart rate	1.76	0.10	1.31	0.23	2.58	0.03
Oxygen consumption	0.31	0.50	0.41	0.50	0.79	0.44
Carbon dioxide production	0.25	0.50	0.07	0.50	0.23	0.50
Respiratory quotient	0.00	0.50	2.34	0.05	2.98	0.01
Total ventilation	0.18	0.50	1.78	0.10	2.04	0.07

The greater blood lactate response to submaximal exercise of the unfit subject as compared with the fit person is also seen in the case of the stool stepping exercise (type II) with varying loads. In this case an extremely unfit subject (P. L.) was compared with a man who was clearly above average in the matter of exercise tolerance. From the results (fig. 5) it will be seen that P. L. carrying no load gave a much greater lactate response than R. S. with either no load or with a 10 lb. load. It will also be seen that P. L. with only a 10 lb. load gave a greater response than did R. S. with 50 lbs. The heart rate for these two subjects (maximal exercise rate and rate 1 minute after exercise) also indicates clearly the difference in fitness between these two individuals (fig. 5).

DISCUSSION AND INTERPRETATION. The time course of blood lactate to submaximal exercise confirms the interpretation of Bang (1936) that lactate production, beginning immediately after the onset of exercise, rises to a maximum, and then declines without attaining a steady level for any significant period of time. No equilibrium conditions are apparently set up between the processes of production and dissipation of lactic acid; rather this compound appears to be related to a period of adjustment associated with the beginning of activity.

Heretofore inadequate data or failure to apply rigorous statistical methods have left the problem of lactate and physical fitness in an inconclusive state. The findings of Robinson and Harmon (1941) and of Knehr, Dill and Neufeld (1942), on the effects of training, are extremely suggestive, but the variations between individuals appear to have been so great that it is unfortunate a statistical treatment of the data was not published. The results of blood lactate de-

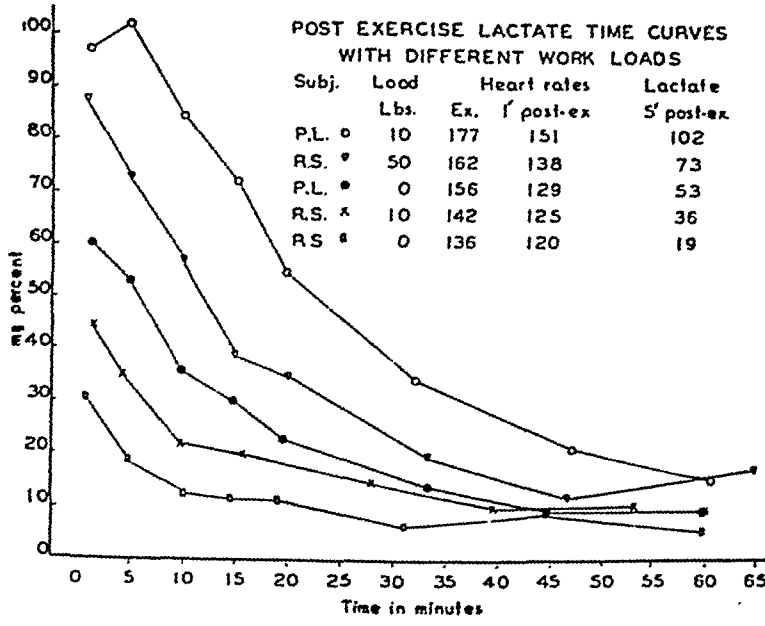

Fig. 5

terminations on 40 men after a treadmill run (Johnson and Brouha, 1942) are also available in this connection. These authors segregated their subjects into 4 groups on the basis of an estimate of relative fitness and from the results concluded that the blood lactate is higher in the groups lower in the scale of fitness. Actually this is what the present report indicates in so far as submaximal work is concerned, but it is difficult to see how the data of Johnson and Brouha prove this point. Not only is group D (men in poor condition) misplaced (since the data show the mean blood lactate to be 113 mgm. per cent, rather than 131 and 130 as given by Johnson and Brouha), but no statistically valid difference can be shown to exist between the two extreme groups (A and D). In reality Johnson and Brouha's experimental arrangement was a poor one to decide the relationship between lactate and fitness, since men able to run for five minutes were stopped at that time, while others continued to the exhaustion point because this was still

within the five minute limit. This arrangement tends to make the work submaximal for groups A and B whereas it is exhaustive for groups C and D. As Robinson and Harmon (1941) have pointed out, the lactate response is quite different in the two types of activity.

One suggestion that emerges from the results of table 2 is the finding that the total excess urine lactate is, like the blood lactate, related to fitness in the case of submaximal work. The training studies of Lewis, Hewlett and Barnett (1925) also point to the same conclusion. Practically, the use of urine in a test for physical fitness would not only obviate the problem of blood collection but would also yield a large quantity of material for analysis, making unnecessary micromethods with their attendant difficulties and errors. The use of urine in connection with a macro-method might greatly improve the figures for the reliability coefficient of a lactic acid determination.

SUMMARY

Experiments are reported in which the blood and urine lactate concentrations were determined in rest, during work of various types, and in the recovery period following this work. The response of 19 subjects in varying degrees of fitness to a 15 minute treadmill walk (submaximal work) indicates that the blood lactate rises rapidly with the onset of work, reaches a maximum concentration at or before the completion of the work and then declines along a die-away curve, reaching the resting level in from 30 to 90 minutes after the end of the walk. As a group, less fit individuals appear to give a significantly greater blood lactate concentration throughout the entire period of the lactate response to the exercise than do fit individuals. Lactate excretion via the urine is maximal in the 10 to 20 minute period after the work and is complete within the 40 to 50 minute period. The total excess urine lactate in response to the activity also is significantly related to the fitness of the group, as less fit individuals, again as a group, show larger quantities of this compound. In a test-retest series on 31 subjects involving both submaximal and maximal work it was found that the reliability of blood lactate determinations is at least as high as the reliability of other commonly used physiological measures.

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THE EFFECT OF SOME INTERNAL FACTORS ON HUMAN WORK OUTPUT AND RECOVERY[1]

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Received for publication March 11, 1944

The idea has been held by those interested in the study of fatigue that whether a subject feels "good" and "full of pep" or whether he feels "bad" and "tired" has no relation to the amount of work he is capable of performing on an ergometer. This idea apparently had its origin in observations to the effect that when a subject felt good, his work output was not always increased, and when he felt bad it was not always decreased.

It appeared that no data had been analyzed to determine the relationship between these factors. It is quite conceivable that feeling good might actually be associated with decreased work output on the ergometer. Therefore, these data were collected and analyzed. In addition, we collected and analyzed data regarding the relationship of the amount of sleep obtained by the subject in the preceding twenty-four hours and also of the period which such sleep covered.

The *internal factors* referred to in the title of this paper have not been further analyzed by us; we have not even attempted to name them. For instance, there exists some factor which will cause a given subject on a given day to exceed his average work output in the first work-period and to compensate for it by a lower work output in the second work-period, thus producing a low percentage of recovery. We have chosen to call these unknowns *internal factors* by way of contrast with such external factors as the environmental temperature and humidity.

METHOD. Seven trained subjects (medical students) performed double work-periods on the bicycle ergometer (1). A total of 378 such performances comprise the data of the present study. These data are compared with the following: the subject's estimate of his feeling-tone at the beginning of the performance, the amount of sleep obtained by him in the preceding twenty-four hours, and the period which such sleep covered.

In the selection of subjects, muscular development was given no consideration; willingness on the part of the candidate to co-operate in the investigation constituted the sole basis of selection. The subject's physical exercise was limited to his performances on the ergometer. His diet was adequate in all known components and food intake was a constant factor. The subjects were required to eat and sleep in a hospital near the laboratory.

[1] This study was aided by a grant from the Abbott Fund of Northwestern University. The authors are greatly indebted to Mr. Kenneth W. Revell and Mr. Daniel H. Fillmore of the United States Public Health Service for access to, and assistance in the use of, statistical machinery for punching and sorting cards, and to Dr. A. C. Ivy for the initial inspiration.

The performance on the ergometer consisted in the following: the subject performed until unable to maintain a rate of 1,235 kgm.-m. per minute with a pedalling rate of 54 revolutions per minute, then rested for ten minutes on a nearby bed, and afterwards performed again until unable to maintain the stand-

TABLE 1

First riding time in relation to recovery

FIRST RIDING TIME	PERCENTAGE OF RECOVERY											
	30-39	40-49	50-59	60-69	70-79	80-89	90-99	100-109	110-119	120-129	130-139	Totals
minutes												
1.00- 2.99				3	17	20	4	2	1		1	48
3.00- 4.99		2	22	42	31	8						105
5.00- 6.99		2	11	60	42	10	4	1				130
7.00- 8.99		6	27	22	5							60
9.00-10.99	1	12	9	1								23
11.00-12.99	2	5	2									9
13.00-14.99		3										3
Total	3	30	71	128	95	38	8	3	1	0	1	378

TABLE 2

Total riding time in relation to recovery

TOTAL RIDING TIME	PERCENTAGE OF RECOVERY											
	30-39	40-49	50-59	60-69	70-79	80-89	90-99	100-109	110-119	120-129	130-139	Totals
minutes												
2.00- 3.99				2	7	15	2	2	1			29
4.00- 5.99			2	23	25	6	2				1	59
6.00- 7.99		2	20	15	11	3						51
8.00- 9.99		1	7	43	21	8						80
10.00-11.99		4	10	29	26	5	3	1				78
12.00-13.99		6	21	13	1	1	1					43
14.00-15.99	2	9	6	2	4							23
16.00-17.99	1	4	3	1								9
18.00-19.99		2	2									4
20.00-21.99		2										2
Totals.....	3	30	71	128	95	38	8	3	1	0	1	378

ard rate of performance. The use of the double work-period thus described has been found to give more consistent results (2). The time of performance was on Monday, Wednesday and Friday afternoons between two and four o'clock. Record of the performance was in terms of minutes and seconds for first riding time and for second riding time.

The subject's estimate of his feeling-tone at the beginning of the performance

was provided at that time by means of a scale of 15 points, with *1* representing "the worst you ever felt in your life," *8* "the way you usually feel," and *15* "the best you ever felt in your life." At the same time the subject indicated the

TABLE 3

Feeling-tone in relation to total riding time

INDEX OF FEELING-TONE	TOTAL RIDING TIME (MINUTES)										
	2.00-3.99	4.00-5.99	6.00-7.99	8.00-9.99	10.00-11.99	12.00-13.99	14.00-15.99	16.00-17.99	18.00-19.99	20.00-21.99	Totals
4			1	2							3
5	1		2		1	1					5
6	1	9	5	3	1		2	1			22
7	6	5	3	6	7	5		2			34
8	4	27	32	38	41	14	6	3	1		166
9	5	5		3	5	2	1		1		22
10	5	11	2		2		1				21
11	6	1									7
12	1	1					1		1		4
No infor.			6	28	21	21	12	3	1	2	94
Totals.....	29	59	51	80	78	43	23	9	4	2	378

TABLE 4

Number of hours of sleep in relation to total riding time

NUMBER OF HOURS OF SLEEP	TOTAL RIDING TIME (MINUTES)										
	2.00-3.99	4.00-5.99	6.00-7.99	8.00-9.99	10.00-11.99	12.00-13.99	14.00-15.99	16.00-17.99	18.00-19.99	20.00-21.99	Totals
1.00- 1.99				1							1
2.00- 2.99											0
3.00- 3.99				2							2
4.00- 4.99								1			1
5.00- 5.99	2		2	1	4			1			10
6.00- 6.99		9	8	6	15	5	3				46
7.00- 7.99	11	24	6	27	25	12	8	4	3		120
8.00- 8.99	14	26	29	16	13	5	1				104
9.00- 9.99	1			1							2
10.00-10.99	1										1
No infor.			6	26	21	21	11	3	1	2	91
Totals. ..	29	59	51	80	78	43	23	9	4	2	378

amount of sleep obtained by him in the preceding twenty-four hours and the period which the sleep covered, by stating the bed-time and the waking-time.

The data of performance on the ergometer have been analyzed as follows: on the one hand, in terms of the number of minutes comprising, respectively, the *first riding time*, the *second riding time* and the *total riding time*, or sum of the first

and second riding times; on the other hand, in terms of the quotient obtained on dividing one hundred times the second riding time by the first riding time which is designated the *percentage of recovery*.

TABLE 5

Bed-time in relation to total riding time

BED-TIME	TOTAL RIDING TIME (MINUTES										
	2.00–3.99	4.00–5.99	6.00–7.99	8.00–9.99	10.00–11.99	12.00–13.99	14.00–15.99	16.00–17.99	18.00–19.99	20.00–21.99	Totals
22:00–22:29	1			1							2
22:30–22:59				6	6						12
23:00–23:29	5	8	4	15	5	4	1				42
23:30–23:59	9	18	28	16	16	4	3	2	2		98
24:00–24:29	9	20	2	10	19	6	2	2	1		71
24:30–24:59	2	4	5	2	5	6	6				30
1:00– 1:29	3	6	5		2	2					18
1:30– 1:59		1	1		3			1			6
2:00– 2:29		2		1							3
2:30– 2:59				2	1						3
3:00– 3:29								1			1
3:30– 3:59				1							1
No infor.			6	26	21	21	11	3	1	2	91
Totals.....	29	59	51	80	78	43	23	9	4	2	378

TABLE 6

Feeling-tone in relation to recovery

INDEX OF FEELING-TONE	PERCENTAGE OF RECOVERY											
	30–39	40–49	50–59	60–69	70–79	80–89	90–99	100–109	110–119	120–129	130–139	Totals
4			1		2							3
5			1		2	2						5
6		2	1	5	8	2	2		1		1	22
7		4	3	9	11	7						34
8	1	9	31	65	49	6	3	2				166
9		1	1	8	7	4	1					22
10			2	11	2	5	1					21
11				1	1	4		1				7
12		1	1		1	1						4
No infor.	2	13	30	29	12	7	1					94
Totals...	3	30	71	128	95	38	8	3	1	0	1	378

The data of the subject's estimate of his feeling-tone at the beginning of the performance have been analyzed in terms of the *index of feeling-tone* according to the scale used; the amount of sleep obtained by him in the preceding twenty-four hours, in terms of the *number of hours of sleep*, and the period which the sleep covered, in terms of *bed-time* according to half-hour intervals.

RESULTS. 1. It is here shown that the percentage of recovery is negatively correlated with the sum of the work done in the two work-periods (table 2). This is the quantitative confirmation of an inference which can be drawn from chart

TABLE 7

Number of hours of sleep in relation to recovery

NUMBER OF HOURS OF SLEEP	PERCENTAGE OF RECOVERY											
	30-39	40-49	50-59	60-69	70-79	80-89	90-99	100-109	110-119	120-129	130-139	Totals
1.00- 1.99					1							1
2.00- 2.99												0
3:00- 3.99				1	1							2
4.00- 4.99		1										1
5.00- 5.99			2	3	4				1			10
6.00- 6.99	1	4	4	15	16	4	2					46
7.00- 7.99	1	9	16	44	37	9	4					120
8.00- 8.99		3	21	34	24	18	1	2			1	104
9.00- 9.99				2								2
10.00-10.99								1				1
No infor.	1	13	28	29	12	7	1					91
Totals.	3	30	71	128	95	38	8	3	1	0	1	378

TABLE 8

Bed-time in relation to recovery

BED TIME	PERCENTAGE OF RECOVERY											
	30-39	40-49	50-59	60-69	70-79	80-89	90-99	100-109	110-119	120-129	130-139	Totals
22:00-22:29				1				1				2
22:30-22:59			1	7	4							12
23:00-23:29		2	1	16	15	8						42
23:30-23:59	1	5	26	34	18	12	1	1				98
24:00-24:29		4	5	27	25	6	3	1				71
24:30-24:59	1	4	8	7	7	1	2					30
1:00- 1:29		1	1	2	8	4	1		1			18
1:30- 1:59			1	2	3							6
2:00- 2:29				1	1						1	3
2:30- 2:59				2	1							3
3:00- 3:29		1										1
3:30- 3:59					1							1
No infor.	1	13	28	29	12	7	1					91
Totals.	3	30	71	128	95	38	8	3	1	0	1	378

5 in a previous publication (3) to the effect that when the total work output of a given performance is increased above that of previous performances, it concerns mainly the first work-period. The work output of the second work-period remains fairly constant. This leads to the conclusion that training increases the

ability to do work in the first work-period and does not greatly increase the ability to recover (table 1).

2. There is a slight but significant negative correlation between feeling-tone and the total work output (table 3); the better a subject said he felt, the less his total work output. There is no correlation of feeling-tone with percentage of recovery (table 6).

3. There is a somewhat stronger negative correlation between the amount of sleep and the total work output (table 4); the more sleep a subject has had on a given night, the less his total work output the next day. This agrees with the findings of Laslett (4). No relation of sleep to percentage of recovery is seen (table 7).

4. There is no evident relation of bed-time on a given night to the amount of work (table 5) or percentage of recovery (table 8) on the following day.

SUMMARY

Trained human subjects performed double work-periods on the bicycle ergometer, and the quotient obtained on dividing one hundred times the amount of work in the second period by the amount of work in the first period was called the percentage of recovery.

At the beginning of each performance the subject provided estimate of his feeling-tone at that time, and statement of his bed-time and waking-time in the preceding twenty-four hours.

Comparisons of data have provided the following indications of relationship:

	Total work output		*Percentage of recovery*	
	TABLE	*r*	TABLE	*r*
First work output			1	−0.62
Percentage of recovery	2	−0.53		
Feeling-tone	3	−0.12	6	0.00
Hours of sleep	4	−0.25	7	+0.07
Bed-time	5	+0.04	8	+0.06

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EFFECT OF MANGANESE INTAKE UPON CONCENTRATION OF BISULFITE-BINDING SUBSTANCES IN BLOOD[1]

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Received for publication April 10, 1944

The fact that manganese ions activate numerous enzyme systems *in vitro* (1–15) indicates that this element functions in the animal body to a great extent through its effect upon enzymatic activity. Supporting this idea is the demonstration by Combs *et al.* (16) working with chicks, of a relationship between the manganese content of the diet and phosphatase activity of bone. Wachtel *et al.* (17), working with rats, found that blood phosphatase but not bone phosphatase was related to manganese intake. According to Shils and McCollum (18) and Boyer *et al.* (19) manganese deficiency reduces arginase activity of the liver. In view of the pronounced effect of manganese ions upon cocarboxylase activity *in vitro* (1–3) it appears probable that in extreme manganese deficiency an impairment of carbohydrate metabolism may result. Accordingly a study was made of the activation of cocarboxylase *in vivo* as measured by concentration of bisulfite-binding substances in the blood of rats on low, medium and high intakes of manganese.

PROCEDURE. Animals with limited reserves of manganese were prepared by placing stock colony rats with their litters, when the latter were two weeks of age, in cages provided with wire false bottoms and feeding them a milk-iron-copper ration until the young weighed 40 to 50 grams. The litters were then divided so as to provide a litter-mate control of the same sex and approximately the same weight for each animal receiving a manganese supplement.

Three basal rations low in manganese were used. Ration I consisted of whole milk supplemented with 1.0 mgm. of iron and 0.1 mgm. of copper per 100 cc. Upon analysis of a composite sample of the milk representing several days' deliveries it was found to contain 0.053 mgm. of manganese per liter. Ration II differed from the first only in that glucose was added at the rate of 5 grams per 100 cc. In view of the response *in vitro* (3, 20) of cocarboxylase to magnesium the third ration was planned to furnish a suboptimal intake of this element. It contained 59 p.p.m. of magnesium and had the following percentage composition: crude casein 18, sucrose 75.7, salts[2] 5, fortified corn oil[3] 1, and a mixture[4] of B

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

[2] Except for omission of magnesium and manganese compounds this salt mixture was essentially that of Phillips and Hart. J. Biol. Chem. 102: 657. 1935.

[3] Carotene 0.133 gram, viosterol 1 gram, alpha-tocopherol 0.4 gram, 2-methyl-1, 4-naphthoquinone 10 mgm., corn oil 100 grams.

[4] Thiamine hydrochloride 20 mgm., pyridoxin hydrochloride 20 mgm., riboflavin 50 mgm., calcium pantothenate 0.1 gram, nicotinic acid 5 grams, p-aminobenzoic acid 5 grams, inositol 10 grams, choline chloride 10 grams.

vitamins 0.3. On the basis of solids the three rations contained 0.4, 0.3 and 1.5 p.p.m. of manganese, respectively. Addition of the respective manganese supplements to the three rations raised the levels of manganese approximately to 8, 6 and 500 p.p.m. The paired feeding method was employed with rations I and III; the other ration was fed *ad libitum*. The animals were maintained on the experimental rations about 12 weeks. Two days before the rats receiving ration I were killed they were fed ration II *ad libitum* so as to increase carbohydrate

TABLE 1

Bisulfite-binding substances in blood of rats*

PAIR NO.	CONTROL	Mn-FED	Mn-FED/CONTROL
Ration I (milk-iron-copper)			
	mgm. per 100 cc.	*mgm. per 100 cc.*	*per cent*
1	7.35	7.39	101
2	9.69	8.12	84
3	12.67	8.51	67
4	7.18	8.01	112
5	7.84	5.75	73
6	6.93	7.94	115
7	6.38	7.53	118
8	6.52	6.58	101
9	6.80	7.35	108
10	6.68	6.30	94
11	8.88	7.64	86
12	5.78	6.41	111
13	6.91	5.78	84
14	5.88	7.90	134
Average	7.54	7.23	96

PAIR NO.	CONTROL	Mn-FED	Mn-FED/CONTROL
Ration II (milk-iron-copper-glucose)			
	mgm. per 100 cc.	*mgm. per 100 cc.*	*per cent*
15	9.58	9.30	97
16	8.40	9.74	116
17†	6.32	6.64	105
18†	7.88	7.12	90
19‡	5.36	6.07	113
20‡	5.42	4.85	89
Average	7.16	7.29	102
Ration III (low magnesium)			
21	8.63	9.93	115
22	8.25	8.04	97
23	11.42	11.09	97
24	8.61	10.44	121
25	7.14	7.46	104
Average	8.81	9.39	107
Grand average	7.70	7.68	100

* Calculated as pyruvic acid.

† Fed 5 cc. of ration per 100 grams weight 2 hours before being killed.

‡ Fed 0.5 gram glucose per 100 grams weight by stomach tube 2 hours before being killed. Except where indicated the animals were fasted 12 hours before being killed.

intake. Animals on rations I and III were fasted for 12 hours before being killed. Treatments of animals reared on ration II were varied somewhat as is indicated in footnotes to table 1.

Blood samples were obtained by decapitation of the animals after being stunned by a blow on the head. Bisulfite-binding substances in the blood were determined by the method of Wilkins, Taylor and Weiss (21).

RESULTS. In table 1 it will be noted that considerable variations in concentration of bisulfite-binding substances were found among the bloods of a given

group. For example, in the control group on ration I the extremely high value of 12.67 mgm. per 100 cc. is more than twice the lowest value. The average concentration for this group, 7.54 mgm. per 100 cc., approximates that of the second group of control animals, which received the glucose supplement throughout the entire growth period. The average for the control animals on ration III, 8.81 mgm. per 100 cc., is somewhat higher than that of either of the other control groups. When the concentrations of bisulfite-binding substances in the blood of the manganese rats are compared with those of the respective control animals, it will be seen that there was no consistent difference between manganese blood and control blood. On ration I the concentrations of bisulfite-binding substances averaged 7.54 and 7.23 mgm. per 100 cc., respectively, in the blood of the control and manganese-supplemented animals. On ration II the controls averaged 7.16 whereas the manganese group averaged 7.29. The averages were 8.81 and 9.39, respectively, for the animals receiving ration III alone and when supplemented with 500 p.p.m. of manganese. The grand average for all control animals was 7.70 as compared with 7.68 for their mates which were fed manganese. In column 4 it will be noted that the concentrations of bisulfite-binding substances in blood of the manganese animals ranged from 67 to 134 per cent of those found in the control bloods. Of the 25 pairs of rats upon which determinations were made there were 14 in which the higher concentrations of blood bisulfite-binding substances were found in the manganese-fed animals.

SUMMARY

Bisulfite-binding substances were determined in blood of rats grown on three low-manganese rations with and without manganese addition.

The concentration of bisulfite-binding substances in blood of rats maintained on rations containing 0.3 and 0.4 p.p.m., respectively, of manganese was not affected by a twenty-fold increase in the intake of this element.

Rats consuming a ration low in magnesium as well as in manganese contained in their blood a higher average concentration of bisulfite-binding substances than those fed a milk diet. This condition was not altered by supplementing the ration with a high intake of manganese, 500 p.p.m.

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RESPONSE TO CHILLING AND RECOVERY IN ADRENALECTOMIZED CATS[1]

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Received for publication April 17, 1944

The characteristics of adrenal insufficiency are best demonstrated in adrenalectomized animals which have been maintained on adequate adrenal extract until their wounds have been completely healed and they are in a good state of nutrition. Then changes that develop from insufficient treatment are due solely to the lack of hormones, the operation itself playing no part.

Stresses accentuate adrenal insufficiency so that it may be more easily studied. Animals prepared in the above manner are ideal for such observations, while cold is one of the stresses which readily distinguishes the adrenal insufficient animal from the normal one. This has been shown in rats to be due, at least partially, to the inability to produce the increased amount of heat required (1, 2). Adequate treatment with extract enables them to produce this heat (3). It appears that the failure to increase their heat production is only one of the factors in the greater sensitivity of adrenalectomized animals to cold. The present investigation was conducted in order to discover other factors involved.

We have followed many of the changes which developed during the exposure to cold, as well as those that occurred during recovery. Cats have been used because they lend themselves to certain determinations better than do rats. Blood pressure and heart rate indicate the state of the circulatory system. The volume per cent of erythrocytes is a measure of the water shift to or from the blood. Blood sugar determinations give a partial insight into the carbohydrate metabolism. The plasma electrolytes have been determined since Barbour et al. (4) found a change in monkeys exposed to cold.

By following the course of recovery after exposure to the low temperature, we discovered that some individuals collapsed and died several hours after the body temperature had returned to normal. This was apparently due to a more rapid temperature rise.

METHODS. Healthy adult cats that had been kept in the laboratory for several weeks were used for these observations. They were fed beef heart, canned salmon and fresh milk. Adrenalectomy was performed by the lumbar pathway in two stages, from a week to several months apart. No adrenalectomized animals were employed for the final experiment until the wounds had completely healed. Maintenance of appetite and body weight indicated that the treatment with adrenal extract was adequate before exposure to cold. The last injection was given, in most instances, 16 to 24 hours before the experiment started. From our previous experience we know that this would furnish insufficient protection against a stress.

[1] Aided by a grant from the Comly Fund of Ohio State University.

Blood was taken from either the pinna or the femoral vein for sugar determinations, while it was taken from the latter vein for the other determinations. Blood pressure was determined by means of a mercury manometer connected to the femoral artery by a hypodermic needle. Dissections and manipulations of these vessels were carried out under local novocain anesthesia.

The animal was cooled by wetting the fur or by clipping the hair, and then placing in a room at +3°C. On completion of chilling the animal was dried and placed near an electric heater, care being taken to prevent overheating. It required 4 to 5 hours for the rectal temperature to return to normal, except in some of the later experiments when the rate was slower.

Sugar was determined by Somogyi's modification of the Shaffer-Hartmann method (5); sodium by the method of Salit (6); and chloride by the adsorption indicator method of Saifer and Kornblum (7).

TABLE 1

Reaction of normal cats to cold

CAT	EXPOSURE	RECTAL TEMP.	BLOOD PRESSURE	ERYTHROCYTES	PLASMA Na	PLASMA Cl
	hours	*°C.*	*mm. Hg.*	*volume per cent*	*m.Eq./l.*	*m.Eq./l.*
AE (3.00 kgm.)	4.7	37.5	126	33.0	154	127
		28.5	107	32.3	153	121
BR (1.51 kgm.)	4.3	36.0	115	32.0	151	125
		27.5	71	29.1	149	128
BV (3.2 kgm.)	3.0	37.0	119	44.6	151	125
		29.0	127	45.3	151	122
CW (1.8 kgm.)	5.7	37.5	133	27.4		
		25.0	103	27.2		
Average	4.4	Fall of 9.5	Fall of 21.3			

Sex appeared to make no difference in the results; therefore it is not reported. Medium-sized animals were used, since it was impossible to lower the temperature in large animals without great difficulty.

RESULTS. *Body temperature.* There was a greater fall in body temperature in the adrenalectomized than in normal animals, even with considerably less exposure to cold. Thus, the average rectal temperature of four normal animals with an average exposure of 4.4 hours was 27.5°C., an average fall of 9.5°, while the average rectal temperature of eight adrenalectomized cats exposed for an average of 2.1 hours was 25.25°C., an average fall of 11.4° (tables 1 and 2).

Blood pressure and pulse rate. The arterial pressure fell lower in the adrenalectomized cats than in the normal ones. The average change for the normal cats was from 123 to 102 mm. of mercury, an average fall of 21.3 mm.; while that for the adrenalectomized cats was from 128 to 78 mm. of mercury, an average fall of 49.4 mm. There was considerable reduction in the pulse rate in adrenalectomized cats except in one animal (CL) in which there was a rise. The change for six cats was from an average of 194.8 to an average of 77.8

TABLE 2

Reaction of adrenalectomized animals to cold

CAT	EXPOSURE	RECTAL TEMP.	PULSE RATE PER MINUTE	BLOOD PRESSURE	ERYTHROCYTES	BLOOD GLUCOSE		REMARKS
	hours	°C.		*mm. Hg*	*vol. per cent*	*mgm. per cent*		
CA* (2.24 kgm.).....	1.50	35.0‡	165	113	32.5	Before	79	
		29.0§	96	53	33.0	1.5 hr. after	47	Recovered
CL (2.70 kgm.).....	1.75	38.0	160	150	36.2	Before	101	
		30.0	180	94	32.7	2 hr. after	50	Recovered
CR* (2.00 kgm.).....		37.0	200	126	27.5	Before	89	
	0.8	28.0	180	108	26.2		177	
	2.50	21.0	60	78	26.5	Just after	117	
						2 hr. after	60	Recovered
BZ* (2.6 kgm.).....	2.75	36.0	180	134	33.0	Before	77	
		24.0	96	85	41.7	19 hr. after	62	Recovered
BY* (2.9 kgm.).....	2.00	35.0		139	32.0	Before	70	
		27.0		82	32.4	Just after	70	Died in less than 24 hrs.
CF (2.0 kgm.).....	1.66	38.0	216	113	36.8	Before	81	
		26.5	60	76	36.1	Just after	57	Died in about 24 hrs.
CN*† (2.0 kgm.).....	1.50	37.0	200	123	28.2	Before	85	
		25.0	98	89	24.6	6 hrs. after	96	
						10 hrs. after	64	Died in 12 hrs.
CE* (3.1 kgm.).....		38.0	208	121	43.0	Before		
	3.10	20.5	57	67	38.1	Just after	58	Died in 22 hr. (see text)
Average...	2.10	Fall of 11.4		Fall of 49.4				

* No extract administered for 16 hours before exposure.
† Little food eaten last few days.
‡ Before.
§ Immediately after.

beats per minute. This was determined in only one normal animal (CW), where it fell from 264 to 150 per minute. The development of these changes was followed, at hourly intervals, in one adrenalectomized cat. In this the blood pressure fell early. The values in the order: before, after 2 hours, after 3 hours and after 4 hours' exposure, were as follows:—rectal temperature, 38.0, 33.0, 30.0 and 26.0°C.; blood pressure, 146, 93, 74 and 70 mm. Hg; pulse rate, 210, 204, 162, and 102 beats per min.; erythrocytes, 31.0, 28.2, 32.7 and 30.5 vol. per cent; blood sugar, 107, 122, and 78 mgm. per 100 cc. (4th hr. undetermined).

Extract injected a short time before exposure to cold appeared to delay the fall in blood pressure. In one cat (BW, table 3) extract was injected one hour

TABLE 3

Reaction of adrenalectomized cats to cold

CAT	EXPOSURE	RECTAL TEMP.	PULSE RATE PER MIN.	BLOOD PRESSURE	BLOOD SUGAR
	hours	*°C.*		*mm. Hg.*	*mgm. per cent*
AU (2.3 kgm.)	2.7	37.2	228	133	94.2
		25.5	88	78	
7 days later	4.0	38.0	146	210	107 (struggled)
		26.0	70	102	78
BW (1.7 kgm.)	3.0	35.5	192	134	70.5
		28.0	128	90	52
13 days later	1.6	36.4	200	100	84
		29.0	120	70	
CB (2.45 kgm.)	1.75	37.0	208	113	143 (excited)
		29.0	132	78	91
3 days later	3.0	37.0	200	113	103
		27.0	192	70	77

before exposure to cold, during the first experiment. Compared with a subsequent test when the last extract was injected 16 hours before exposure, the blood pressure was higher, fell less, and longer exposure to cold was required to produce nearly the same fall in temperature.

Water shift. Water shift between the blood and tissues, as shown by samples taken before and after exposure to cold, appeared to vary in different individuals. The changes in the volume per cent of erythrocytes in the adrenalectomized animals indicated that there was significant dilution of the blood in three (CL, CN and CE), concentration in one (BZ) in which the sample was taken 19 hours after removal from the cold, and little change in the other four. We made no determinations at a later time.

Plasma electrolytes. The sodium and chloride concentrations in the adrenalectomized animals before and after exposure to cold were as follows: Cat CA—sodium, 143.0 and 143.4 m.Eq./l; chloride, 118 and 117 m.Eq./l. Cat BY—

sodium, 145.6 and 147.4 m.Eq/l.; chloride, 123 and 123 m.Eq./l. Cat CF—sodium, 147.4 and 144.7 m.Eq./l.; chloride, 121 and 121 m.Eq./l.

It will be noted that the plasma electrolytes were lower than normal, indicating an inadequate dosage of sodium factor. However, it will also be seen that no change was shown at the end of the cooling period. Likewise, our normal animals showed no significant changes in plasma electrolytes (table 1) despite a longer cooling period.

Blood sugar. If one follows the blood sugar in adrenalectomized cats, during and after exposure to cold, the changes resemble those occurring in normal animals. In cat CR there was an increase to 177 mgm. after 50 minutes' exposure, then a drop to 117 after 2.5 hours' exposure, with a further fall occurring 2 hours later; while in cat CN there was an increase from 85 to 96 mgm. 6 hours after the exposure, with a fall 4 hours later (table 2). However, the rises in normal animals were usually greater. In three normal cats exposed to cold in a similar manner, the rises were as follows: cat A, rectal temperature 34.5°; 174 mgm. from 83 mgm. per 100 cc.; cat B, rectal temperature 30.0°C.; 190 mgm. from 79 mgm. per 100 cc.; cat C, rectal temperature 32.5°C.; 103 mgm. from 92 mgm. per 100 cc. Blood sugar determinations in adrenalectomized cats after exposure to cold sometimes showed considerable reduction (table 2). Yet there was no greater fall in animals which died than in those that recovered; at least, at the time the determinations were made.

In four normal cats the blood sugar was followed after the exposure. The animals had been fasted for the same period as were the adrenalectomized animals, viz., 24 hours. They were cooled to a rectal temperature of 25°C. by 5 to 10 hours' exposure to 3°C. The blood glucose fell to 25, 28 and 37 mgm. per 100 cc. in three, but rose to 168 mgm. in the fourth. (When this animal was again exposed to cold three weeks later, a fall in body temperature to 24°C. was accompanied by a rise in blood sugar to 265 mgm. per cent.) In this animal it fell to 45 mgm. 4 hours after removal from the cold. These low values are lower than those found in the adrenalectomized cats.

Cerebral activity. The cerebral activity of adrenalectomized cats, whose temperature had been markedly reduced, returned to normal slowly and then sometimes failed. Cat CE was typical. Four hours after removal from the cold the rectal temperature was 29.6°C. and there were no placing reflexes. At 7 hours the rectal temperature was 35.5°C. and the higher reflexes had returned: he purred, drank water, and appeared fairly aggressive, but staggered when walking. At 19 hours, although lying down, he appeared normal. He was dead at 22 hours.

Another adrenalectomized cat, no. 18, was exposed to cold for 2 hours, until the rectal temperature had been reduced to 28.5°C. from 39.0°C. The higher reflexes were poor after two trials following 7 hours in artificial heat, when the rectal temperature had risen to 36.0°C. The knee jerk was absent. After 12 hours he could almost stand, and the blood sugar was 71 mgm. per 100 cc. After 24 hours he was quite active and strong but refused food. Despite one injection of adrenal extract he died 36 hours after removal from the cold.

Effect of repeated exposure. Three adrenalectomized cats which survived

exposure to cold on the first test failed to do so after the second test. The first, cat AU, after 2.7 hours' exposure to cold, showed typical changes (table 3). Recovery was uneventful. Seven days later she was exposed to cold for two hours, taken out for determinations, then replaced in the cold for an hour, taken out a second time for determinations, and replaced for a final hour. The changes are shown in table 3. The animal died two hours after removal from cold while exposed to artificial heat. The pulse rose to 180 per minute and the rectal temperature to 33°C. before death. Arterial blood at death contained 42 mgm. per cent of sugar. Disappearance of cerebral reflexes was the first sign of impending death. The animal had eaten less food than usual since the last test, indicating that it had not completely recovered. However, a longer exposure was required to produce a fall in temperature slightly less than before.

The second cat (BW) made uneventful recovery from the first exposure to cold but 13 days later a shorter exposure caused death. The last extract had been injected 16 hours before exposure while in the previous test an injection had been made an hour before exposure.

Four hours after being removed from the cold room the rectal temperature had reached 37°C. She slept continuously. From the 14th to the 20th hour the placing and hopping reflexes were absent. At the 22nd hour placing reflexes could be elicited once, following which the animal remained unresponsive. At the 24th hour the rectal temperature was 36.2°C. and the pulse rate was 160 per minute. Reflexes were present but the cat staggered when attempting to walk. At 28 hours the rectal temperature was 35.6°C. and the heart rate 160 per minute; the cat was prostrate and cried out upon being moved, as adrenalectomized cats frequently do before they die. The following reflexes were absent: hopping, placing, righting, wink response to threat. The pupil was dilated, barely reacting to light. The pinna reflex and attitudinal reflexes were present. The corneal reflex was slightly exaggerated. Knee jerk, progression reflexes, flexor and extensor tone were exaggerated. Pinching the tail resulted in markedly delayed rigidity and opisthotonos. At 28 hours the lower reflexes became more active, respiration stopped, followed by a tonic convulsion, but the heart continued long enough for the withdrawal of 5 cc. of blood by heart puncture. The blood sugar was quite low, 26 mgm. per cent. The plasma sodium (145 m. Eq./l.) and chloride (121 m. Eq./l.) showed no change.

From this experiment, as well as others, it is evident that the higher nerve centers fail first.

A third cat (CB) also made uneventful recovery from the first exposure (table 3). Three days later exposure to cold was followed by apparent recovery and then death. Nine hours after removal from the cold the cat seemed in good condition and responded normally so that we prophesied his recovery. Nevertheless we continued to watch him because other animals had appeared just as well but were found dead in the morning. At 13 hours the cat had a brief convulsion. He drank a little water when his lips were touched with it. At 13.5 hours he cried out; there was no response to threat, there were no placing reflexes; visual response was gone. He was prostrate, being seized by convulsive jerks.

At 14 hours, the rectal temperature was 36°C., the blood pressure was 70 mm., and the blood sugar (femoral vein) 72 mgm. per cent. Ten cubic centimeters of 5 per cent glucose (anhydrous) was injected slowly into the femoral vein. Five minutes later the cat responded to a threat, and placing reflexes were present but sluggish. In 20 minutes he climbed 15 inches onto a cage where a heater was located. He showed good judgment and co-ordination. Fifty minutes after the sugar injection the blood sugar was 89 mgm. Sixty minutes after injection the blood pressure was still 70 mm. but the threat response was poor and reflexes had become very sluggish. Pupillary reaction to light was also poor. He began to appear restless, shifting position slightly, then lying down—the typical reaction of an impending crisis in adrenal insufficiency.

Sixteen hours after removal from the cold 18 cc. of 5 per cent glucose was injected intravenously. In 3 minutes the cat again jumped upon the cage to be

TABLE 4

Blood sugar of adrenalectomized cats exposed to cold

TIME	JU		KK		KL		KZ	
	Rectal T.	Blood sugar	Rectal T.	Blood sugar	Rectal T.	Blood sugar	Rectal T.	Blood sugar
	°C.	mgm. %	°C.	mgm. %	°C.	mgm. %	°C.	mgm. %
Before	37.8	64	38.2	67	37.6	65	38.2	58
Lowest temperature	23.5	30	25.0	29	24.0	30*	24.7	31
7.5 hrs. after	32.3	26	30.0	26	34.4	46	31.3	30
8 days later								
Before	38.3	63	38.2	65			38.2	70
Lowest temperature	27.2	22†	24.3	11‡			22.3	28

* Taken 2 hrs. after removal from cold.
† Taken 1 hr. after lowest temperature.
‡ Taken 1.5 hr. after lowest temperature.

near the heater. Reflexes were then very prompt. Seventeen minutes after the last injection, reflexes were still very good. The cat licked himself and appeared sleepy. He soon appeared uncomfortable, changing position. The blood sugar was 166 mgm. At 17 hours the cat was unsteady on his hind legs. At 19 hours he responded to threat, the placing reflex was extremely sluggish and the righting reflex was effective. He could stand in a wobbly fashion but cried on being handled. At 19.7 hours he died. The blood sugar obtained from the vena cava before the heart stopped was 77 mgm. per cent.

We watched many of our animals for 24 hours after removal from the cold in an attempt to discover them in a crisis so that injections might be tried, but, in all others observed, the crisis was so brief or the premonition so inadequate that death occurred before anything could be done.

When death occurred, it was usually many hours after removal from the cold, several hours after the body temperature had returned to normal. This was

always at least twenty-four hours after injection of the last dose of adrenal extract. We suspected that the rate of rise of body temperature after chilling was a factor in the failure to recover. Therefore we varied the procedure in 4 adrenalectomized cats by raising their temperature more slowly after exposure to cold.

Effect of slower rise of body temperature. Instead of raising the rectal temperature to normal in 4 to 5 hours, this period was prolonged to 8 to 10 hours. These animals were deprived of extract for 24 hours before exposure to cold. In all other respects the procedure was the same.

The rectal temperature fell to 25°C. or less and the blood sugar to 31 mgm. per 100 cc. or less (table 4). The blood sugar remained low for 7.5 hours after the animals were removed from the cold. No determinations were made later than this. In spite of a longer period without extract all animals made an uneventful recovery. Three of these cats were tested in the same manner 8 days later with similar results, except that the blood sugar fell to still lower levels (table 4).

Discussion. There are two aspects of our experiments, i.e., the primary effect of the exposure to cold, and the recovery following. The former is undoubtedly a factor in the latter, since it depletes the body's store of hormone more rapidly.

The greater ease with which the body temperature was lowered after adrenalectomy in cats is probably due to inability to produce the extra heat required, as in rats (1, 2, 3). One first thinks of the available carbohydrate. The changes in blood sugar indicated no significant difference in this between the treated adrenalectomized animals and normal, either at the end of the exposure to cold or during the crisis of the recovery period. Blood sugar fell to low levels in both groups. Moreover, there was no indication that the fall in blood sugar was greater in the adrenalectomized animals that died than in those that survived. It is interesting to note that, on two different occasions, in one normal animal (LJ) it was possible to lower the body temperature to 25.2 and 24.0°C. respectively with blood sugar values of 168 and 265 mgm. per cent. One adrenalectomized cat (CR) showed a high blood sugar (177 mgm. per cent) when the rectal temperature had fallen to 28°C. and the blood sugar was still high (117 mgm. per cent) when the rectal temperature had fallen to 21°C.

Cat BW which had convulsions at death, unlike most animals, had a blood sugar value of convulsion level since the rectal temperature was near the normal range. Other low blood sugars were present, usually accompanying low body temperature which would preclude convulsions (8).

Often, adrenalectomized cats that survived one cooling failed to survive the second test. One of these (CB) had a blood sugar of 77 mgm. per cent at death (due in part to injected sugar). Therefore, depletion of carbohydrate did not appear to be responsible for the outcome.

The brain is peculiarly susceptible to hypoglycemia since it stores little carbohydrate (9) and seems to depend solely on carbohydrate for its energy (10). Himwich and Fazekas (11) determined the glucose and oxygen utilization of the brain during hypoglycemia. As long as the arterial blood sugar was 46 to 25 mgm. per cent the glucose and oxygen utilization were little reduced. But when the arterial blood sugar fell to values between 22 and 7 mgm. per cent

the glucose absorption decreased to 3 mgm. per cent (from 13.1 mgm.) and that of oxygen to 3.8 vol. per cent (from 9.3 vol.). The intravenous injection of glucose during hypoglycemia restored the oxygen consumption to 9.14 vol. per cent.

Low arterial sugar alone would not account for a decreased glucose and oxygen utilization by the brain in our animals because in only one instance (cat BW) did the venous blood sugar value go as low as 26 mgm. per 100 cc. while the body temperature was nearly normal.

The greater fall in blood pressure in adrenalectomized cats as compared to normal animals following exposure to cold indicated a poorer circulatory adjustment.

There was no reason to believe that the blood volume in our adrenalectomized cats was subnormal. It has been shown in adrenalectomized dogs that the blood volume remains within normal limits when the animal is adequately maintained on adrenal extract, whether the amount of sodium factor is adequate or not (12).

The temporary recovery of cat CB following the intravenous injection of glucose was so prompt that a circulatory effect is suggested. Edema of the lungs (13) and heart (14) has been described in animals dying of hypothermia. Whether or not edema of the brain occurred would be difficult to determine. The concentration of glucose injected was very little above isotonic. Therefore, shift of fluid from the tissues to the blood stream would be very small.

Reduction of the cerebral circulation in the adrenalectomized animals following exposure to cold may have been significant since the arterial pressure frequently fell as low as 70 mm. of mercury. The more rapid rate of "warming" would permit less time for recovery and, at the same time, the increased metabolism attending the temperature rise would require more prompt recovery.

The effect of glucose injection in our experiment is best explained as due to the increased volume of circulatory fluid (estimated 8 per cent at the first injection and 14 per cent at the second injection) so that the blood flow to the brain was increased temporarily. The complete recovery of cerebral activity within twenty minutes after the first injection and within three minutes after the second, larger dose, together with the relatively transient effect, are thus explained. A slower rise of body temperature following chilling was never followed by death in the adrenalectomized animals, even if the chilling was repeated.

It has been shown (15) that adrenalectomized animals are less resistant to heat than are normal animals. Up to the present time these results have not been fully explained. Although the body temperature of our cats was never raised above normal the increase in temperature from 25°C. to 37°C. in four or five hours may have been similar in its effect to an increase of temperature caused by an environment of 39 to 40°C. for a shorter period. Although the above explanation of our results seems plausible one must bear in mind the evidence for primary involvement of the nervous system. Thus Elliott (16) and later Hoskins (17) observed a decrease in irritability of the sympathetic nervous system. Hartman and Lockwood (18) found that adrenalectomy decreased the resistance of reflexes to fatigue.

In Addison's disease mental fatigue may precede other symptoms. The patient may become unable to concentrate and protracted application becomes impossible. The Biot type of respiration, which sometimes occurs, indicates cerebral involvement. Vision may become blurred, hearing dulled, and the whole sensorium may lose its acuteness. Abnormalities in the electroencephalogram have been found (19).

In our experiments, when the animal began to fail after regaining normal temperature, the activity of the higher centers disappeared first until, in some instances, the whole cerebrum was involved as indicated by the decerebrate-like rigidity. Finally, respiration failed. Such a syndrome is characteristic preceding death from adrenal insufficiency.

The problem which remains unsettled is that of the primary cause of circulatory failure. It has been demonstrated, in advanced adrenal insufficiency, that the heart action is inadequate (20) and that vasomotor response is poor. The latter has been shown by the less adequate reaction to hemorrhage (21) and the vascular dilatation in the alimentary canal (22). However, none of these observations seem to disclose the primary defect. It is still possible that this might involve the central nervous system despite the fact that the central nervous changes in our experiments may be explained by failure of the cerebral circulation.

SUMMARY

Adrenalectomized cats maintained on adrenal extract adequate for ordinary laboratory life were chilled until the rectal temperature was about 25°C. Artificial heat was supplied so that the normal rectal temperature was regained in either 5 or 10 hours. A much shorter period of chilling was required to produce the same fall in rectal temperature in the adrenalectomized than in normal cats.

The arterial pressure fell to a lower level in the adrenalectomized animals than in normal animals. The changes in volume per cent of erythrocytes were so varied that no consistent shift in plasma was indicated. There were no significant changes in the plasma sodium or chloride.

The increases in blood sugar upon chilling were usually less in the adrenalectomized cats than in normals. Sometimes there was considerable reduction in the blood sugar with or without a preliminary rise. However, there was no greater fall in the animals which died than in those which survived. Normal cats sometimes showed lower blood sugar values than did adrenalectomized cats.

After chilling, the cerebral activity of adrenalectomized cats returned to normal slowly and then sometimes failed. Evidence is offered that this failure was due to a decrease in cerebral circulation.

Adrenalectomized cats which survived one chilling might fail to recover from a second exposure.

We wish to thank R. R. Durant, A. H. Hegnauer, E. J. Robinson and D. E. Smith for technical assistance.

We are indebted to Dr. G. F. Cartland of the Upjohn Company for the adrenal extract used.

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THE METABOLISM OF ACETONE BODIES AND GLUCOSE IN VITRO AND THE EFFECT OF ANTERIOR PITUITARY EXTRACT

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Received for publication April 20, 1944

It is now generally agreed that acetone bodies have their origin in the liver and that they are in turn utilized by various other tissues The evidence which supports this hypothesis has been summarized in several recent reviews (Stadie, 1940; Soskin and Levine, 1941; MacKay, 1943). Although *in vitro* methods have been extensively employed in studies of acetone body metabolism, the tissues under observation have usually been subjected to a highly distorted environment. The incubating medium employed has consisted of a simple buffered solution to which, in most instances, a single substrate has been added. Even though such preparations provide a useful tool for the elucidation of specific metabolic pathways, little is learned about certain over-all aspects of tissue function during life.

The plan of the present series of experiments was as follows: 1, to determine which of the major body tissues produce acetone bodies and which tissues utilize them under conditions of incubation closely approaching a normal environment; 2, to study, in a similar manner, the metabolism of glucose by these same tissues; 3, to test the effect of anterior pituitary extract on the metabolism of acetone bodies and glucose *in vitro*. The work of Collip et al. (1935) and particularly that of Mirsky (1936) has suggested that the pituitary ketogenic principle enhances ketosis by stimulation of hepatic ketogenesis. Little is known concerning the action of this principle on ketone utilization by peripheral tissues.

METHODS. The animals were male rats of Sprague-Dawley strain, weighing 250 grams or more. Both fed and fasted animals were used. The former received Purina dog chow, while the latter were deprived of food for 48 hours before the experiment.

The incubating medium was serum obtained from the rat before removal of the tissues which were intended for incubation. Rats were anesthetized with nembutal and exsanguinated from the abdominal aorta by direct incision of the vessel. One cubic centimeter of the separated serum was placed into a small flat-bottomed vessel which was designed to fit a standard Warburg manometer. The stoppered vessel was weighed before and after introduction of the tissue slice in order to determine the wet weight of the specimen. The organs were immediately removed from the exsanguinated rat and placed in the ice box until they were sliced 15 to 20 minutes later. Slices were cut free hand to a thickness of approximately 0.3 mm. Serum was used as a lubricant. The excess adhering to the slice was removed by touching to filter paper. The tissues were not floated in saline before introduction into the vessel. Diaphragm and omentum

were trimmed to suitable size and used intact. The weight of samples varied from approximately 10 mgm. in the case of kidney to slightly over 100 mgm. in the case of omentum. The latter tissue consisted almost wholly of fat. The weight of control specimens and those treated with extract was kept as nearly the same as possible. Unless otherwise noted, the gas mixture overlying the serum was 95 per cent oxygen, 5 per cent CO_2. The time of incubation was 2 hours and the temperature 37.5°C.

The pituitary extract and the control extracts made from kidney and muscle contained the equivalent of 0.2 gram of gland in 1 cc. of solution. Finely ground tissue was extracted overnight with N/5 NaOH at 5°C., neutralized with HCl to pH 7.5, and preserved in the frozen state. One-tenth cubic centimeter of

TABLE 1

Production or utilization of acetone bodies and glucose per 100 mgm. of wet tissue

TISSUE	TOTAL ACETONE BODIES (GAMMA PER 100 MGM. TISSUE)—2 HOURS			GLUCOSE (MGM. PER 100 MGM. TISSUE)—2 HOURS		
	No. of rats	Produced	Used	No. of rats	Produced	Used
Liver (fed)	15	80 ±5		5	1.3 ±.09	
(fasting)	19	112 ±6		10	0.8 ±.07	
Kidney cortex (fasting)	7		184 ±42	8	1.0 ±.24	
Brain cortex (fasting)	5		41 ±10	5		0.9 ±.10
Diaphragm (fasting)	6		40 ±11	7		0.2 ±.05
Spleen (fasting)	5		34 ±10	5		0.3 ±.02
Omentum (fasting)	5		6 ±2	5		0.1 ±.03

Acetone bodies are expressed as acetone. The values above with their standard error represent the mean changes which occurred during the 2 hour incubation period.

extract was added to one flask just before incubation while another flask served as an untreated control.

Plain serum incubated in the absence of tissue showed no significant change in acetone body or glucose content both in the presence and absence of added pituitary extract.

Acetone bodies were determined by a micro method previously described (Shipley and Long, 1938). Glucose determinations were made by the method of Folin and Malmros (1929). The protein precipitant in each case was tungstic acid. Analyses were made on serum alone.

RESULTS. *Total acetone body production and consumption.* In every instance, without exception, liver tissue produced acetone bodies. The output was significantly higher from the livers of fasted rats (112 gamma per 100 mgm.) than from those of fed rats (80 gamma per 100 mgm.), although the difference was not as striking as might be expected (table 1). Kidney, brain, diaphragm,

spleen and omentum invariably consumed acetone bodies. These latter tissues and the serum in which they were incubated were taken exclusively from fasted animals in order to insure a sufficiently high initial acetone body content of the serum. The highest rate of utilization was by kidney and the lowest by omentum. The rate of utilization tended to be lower in those preparations where the initial serum acetone body concentration was low. It is of particular interest to note that brain consumed acetone bodies at a rate equal to that of muscle. This rate did not differ greatly from rates of consumption previously reported for various muscle preparations and for intact animals (Stadie, 1940).

Fractional determination of acetone bodies. In several experiments, the production by liver of beta-hydroxybutyric acid and acetoacetic acid (plus acetone) were determined separately. The ratio of acetoacetic acid to beta-hydroxybutyric acid in the fresh unincubated serum from fasting rats averaged 0.6 and

TABLE 2

Fractional determination of acetone bodies

	STATUS OF RATS	NO. OF RATS	GAS MIXTURE	RATIO: AC/B-OXY AFTER INCUBATION	CHANGE: ACETOACETIC ACID	CHANGE: B-HYDROXY-BUTYRIC ACID
					gamma per 100 mgm.	*gamma per 100 mgm.*
Liver slices	Fasted	6	95% O_2	2.8 ±0.3	+193	−29
		5	15% O_2	0.7 ±0.1	+52	+27
		3	Anaerobic	0.1 ±0.1	−48	+21
	Fed	6	95% O_2	2.1 ±0.2	+79	+21
Diaphragm	Fasted	4	95% O_2	0.2	−32	−21
		3	15% O_2	0.2	−13	−7

The effect of alteration in oxygen tension on the ratio of the two fractions and on total output is shown. The ratio of acetoacetic acid to Beta-hydroxybutyric acid averaged 0.6 ±0.05 in the entire series of fasted rats before incubation of the serum.

varied very little from animal to animal (table 2). After incubation, there was a striking rise in the ratio to an average of 2.8. The change could be traced to a marked rise in acetoacetic acid and a slight decrease in beta-hydroxybutyric acid. There had occurred a marked distortion in the relative output of the two fractions. When liver slices from fed rats were used, the final ratio was similar. In this instance, both compounds were produced by the tissue, but acetoacetic acid at a much faster rate than beta-hydroxybutyric acid.

When the oxygen content of the gas mixture was reduced to 15 per cent, the ratio remained approximately normal during incubation. With the atmosphere composed of 95 per cent nitrogen and 5 per cent CO_2, the ratio became abnormally low due to conversion of acetoacetic acid to beta-hydroxybutyric acid. There was no overall incease in acetone bodies under anaerobic conditions.

Separate determinations of the two acetone body fractions in the case of diaphragm revealed that both fractions were utilized. Although separate determinations were not made, it was evident that kidney tissue behaved simi-

larly, inasmuch as the total acetone body content of the medium fell to zero in several instances during the period of incubation.

The effect of anterior pituitary extract on acetone body turnover. The addition of anterior pituitary extract to the incubating medium was followed by a distinct increase in the rate of production of acetone bodies by liver slices (table 3). Control kidney and muscle extracts were without effect. The stimulating action of pituitary extract was most notable in the case of the fed rats, where the increase averaged 46 per cent. The mean increase in the fasted group was 27 per cent, but the statistical reliability of this figure was poor. The poor reliability may be partly due to a greater error in the estimation of acetone body production which would arise from the determination of a small difference between two large

TABLE 3

The effect of anterior pituitary extract on acetone body and glucose production and consumption

	ACETONE BODIES				GLUCOSE			
	No. in group	Pituitary extract	No. in group	Boiled pituitary extract	No. in group	Control kidney and muscle extracts	No. in group	Pituitary extract
								%
Liver production								
(fed)	8	+46% ±8	6	+21 ±5	7	−3% ±4	5	+11
(fasted)	12	+27% ±9			7	+9% ±8	7	−15
Diaphragm consumption (fasted)	6	0%					6	+5
Kidney consumption (fasted)	5	−8%						
Kidney production (fasted)							5	−11

The plus values represent per cent increase of either production or consumption, whichever is being measured, and the minus values indicate decreases in the same. Values of "P" determined from Fishers "t" table are as follows: Acetone body production, liver, fed rats, pituitary extract compared to control extracts: $P < 0.01$. The same, "fasted rats": $P = 0.15$. Pituitary extract before and after boiling: $P = 0.02$.

numbers. Thus, in the case of the fed animals, the original serum content averaged 1 mgm. per cent and rose to 5 mgm. per cent during incubation, while the serum of fasted animals averaged 6 mgm. per cent before incubation and 14 mgm. per cent afterward. Furthermore, the relatively poor increase in acetone body production induced by the extract in the series of fasted rats may possibly have been due to the existing high rate of ketogenesis which was already nearing its limit.

Pituitary extract, which had been heated to boiling for 15 minutes at pH 7.0, was tested for activity. It would be expected that 95 per cent of the ketogenic activity should be destroyed by heating in this manner (Shipley, 1942). The heated extract produced a 21 per cent increase in ketogenesis. This response differed significantly from the 46 per cent increase produced by unheated extract

(table 3). That the activity of heated extract was not entirely due to residual ketogenic principle was evidenced by a lack of stimulating effect of the original extract when added in amounts 5 per cent as large as the standard dose (3 expts.). The residual activity might have been due either to the presence of non-specific ketogenic material peculiar to pituitary extract, or the result of the process of heating which could cause the release of substrates or other substances which facilitate ketone formation.

Pituitary extract had no significant effect on acetone body consumption by muscle and kidney tissue (table 3).

Glucose production and consumption. Liver tissue invariably produced glucose whether the rats had been fasted or fed (table 1). The production averaged 1.3 mgm. per 100 mgm. of tissue in the group of fed rats, and 0.8 mgm. per 100 mgm. of tissue in the fasted group. The glucose production by the liver of fasted rats can be assumed to represent for the most part gluconeogenesis inasmuch as there would be insufficient glycogen present to account for an output of this magnitude. All other tissues, with the notable exception of kidney, utilized glucose. Kidney slices produced 1 mgm. of glucose per 100 mgm. of tissue. The output of carbohydrate by kidney tissue was the more striking in that it exceeded the production by liver tissue from fasting animals. That this glucose resulted from the formation of new carbohydrate may be assumed with fair justification, because the store of glycogen normally present in kidney is very small.

Anterior pituitary extract was without significant influence on the glucose production by liver and kidney or on glucose utilization by diaphragm (table 3).

Discussion. The finding that liver tissue invariably produces acetone bodies and that all other tissues invariably utilize them under conditions of incubation very closely simulating those that exist during life lends further weight to recent concepts of the mechanism of ketosis. The output by the liver may be represented as an algebraic summation of production and interconversion. A decrease in oxygen tension results in decreased total production. High oxygen tension shifts the equilibrium acetoacetic acid $\rightleftarrows$ beta-hydroxybutyric acid toward the left and low oxygen tension toward the right. That the two acetone body fractions are readily convertible one to the other by various tissues is well known. The mechanism of anaerobic conversion of acetoacetic acid to beta-hydroxybutyric acid by tissue slices has been studied by Quastel and Wheatley (1935) and Edson and Leloir (1936). In order to duplicate the behavior of the liver as it functions *in situ*, liver slices must be exposed to a gas mixture containing less than 95 per cent oxygen, because with high concentrations of oxygen, the outflow of beta-hydroxybutyric acid is reversed. The correct oxygen content is not necessarily as low as 15 per cent, even though at this level a near normal ratio is maintained during incubation. A predominant output of acetoacetic acid during life would not be inconsistant with a low ac-ac/B-oxy serum ratio, if tissue utilization of this compound were more rapid than that of beta-hydroxybutyric acid.

The disappearance of acetone bodies in the presence of brain tissue would

suggest that this organ utilizes these compounds. This is contrary to the general belief that brain consumes only carbohydrate. Mulder and Crandall (1942) concluded that the dog's brain does not utilize acetone bodies. Their conclusion was based on their failure to demonstrate an arteriovenous difference in acetone body concentration. It may be calculated, however, that the expected A-V difference would be too small to demonstrate by present methods of analysis. If one assumes that a dog brain weighing 100 grams with a blood flow of 100 cc. per minute consumes 0.33 mgm. of acetone bodies in one minute (the latter calculated from the present data), the change in blood acetone body concentration would be only 0.33 mgm. per cent.

The present experiments offer direct evidence that anterior pituitary extract accentuates ketosis by stimulation of hepatic ketogenesis. It of course may not be concluded with certainty that this effect is due to the specific ketogenic principle of the pituitary. The latter is not available in pure form. Crude extracts contain a multitude of substances which might complicate the results of an *in vitro* study. The evidence which suggests, however, that the specific principle itself is responsible consists of the demonstration that two other tissue extracts were inactive and that heating markedly reduced the activity of the pituitary preparation.

The production of glucose by kidney tissue was unique among the various extrahepatic tissues studied. This is worthy of some note inasmuch as the liver has until recently been assumed to be the sole organ participating in gluconeogenesis. Under the conditions of these experiments, however, the glucose output per unit weight of kidney tissue from the fasting animal actually exceeded that of liver. Wilhelmi (1944) has recently obtained similar results when liver and kidney slices were incubated in serum. In the experiments of Weil-Malherbe (1938) and of Stadie, Zapp and Lukens (1941), it will be noted that kidney slices in the absence of substrate formed fermentable carbohydrate during incubation. Russell and Wilhelmi (1941) showed that kidney slices formed considerable carbohydrate in the presence of certain amino acids. Russell (1942) offered the suggestion that the delayed fall of blood sugar in the eviscerated animal, when the kidneys were left in place, might be due to gluconeogenesis by kidney tissue, and Reinecke (1943) has recently produced excellent evidence that such is actually the case. It would appear therefore that the kidney must be included along with the liver as a source of new carbohydrate in the living organism.

SUMMARY

1. Rat liver tissue incubated in rat serum under aerobic conditions invariably produced acetone bodies whether the rat had been previously fasted or fed. Anterior pituitary extract accentuated this ketogenesis. Heating of the extract markedly reduced its activity. Kidney and muscle extracts were inactive.

2. The equilibrium: acetoacetic acid $\rightleftarrows$ beta-hydroxybutyric acid in the presence of liver tissue was very sensitive to changes in oxygen tension. It would appear that the high concentration of oxygen ordinarily used in tissue slice experiments causes an abnormal shift of the equilibrium to the left.

3. Diaphragm, kidney, spleen, omentum and even brain invariably utilized acetone bodies. The rate of consumption by diaphragm and kidney was not influenced by anterior pituitary extract.

4. Liver and kidney without exception produced glucose, while the latter was utilized by muscle, spleen, brain and omentum. Kidney was a better former of new glucose weight for weight than liver.

I am greatly indebted to Miss Ethel Buchwald for technical assistance.

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THE KIDNEY AS A LOCUS OF FRUCTOSE METABOLISM[1, 2]

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Received for publication April 21, 1944

Goda (1938) and Stewart and Thompson (1941) have shown that slices of kidney tissue will cause the disappearance of fructose with the simultaneous appearance of glucose if the conditions are favorable. Griffiths and Waters (1936) and the author (Reinecke, 1942a) have obtained results that indicate that fructose will prolong the life of the eviscerated animal that still retains its kidneys. Since all this evidence suggests that the kidney has a function of some particular importance in the metabolism of fructose, the experiments reported here were undertaken to explore further the possibility of the existence of such a renal function.

METHODS AND MATERIALS. Male albino rats obtained from Sprague-Dawley, Inc. were used. They were prepared, maintained and abdominally eviscerated in a manner previously described (Reinecke, 1943). The analyses for blood sugar, fructose and lactic acid were made on 0.05 cc. samples of blood that were deproteinized with dilute tungstic acid solution in the proportion used in the fructose method (Reinecke, 1942b). Of the approximately 4.5 cc. of filtrate obtained from the 0.05 cc. of blood, 1 cc. was analyzed for fructose. A second cubic centimeter was diluted with 1.5 cc. of water and then analyzed for total sugar (Reinecke, 1942c). A third portion of 2 cc. was treated with 0.5 cc. of 10 per cent copper sulfate followed by an excess of powdered calcium hydroxide. After the cupric hydroxide and excess calcium hydroxide had been centrifuged down, lactic acid was determined in the supernatant liquid (Barker and Summerson, 1941). One-tenth cubic centimeter samples of blood were taken in many instances to allow the analyses to be checked. The difference between the blood fructose and total sugar was taken as the blood glucose. The methods used for analyzing the carcasses for fructose are detailed in the legends of the respective figures.

RESULTS. *The destruction of fructose by the nephrectomized and the non-nephrectomized, eviscerated rat.* (See fig. 1.) It was found that if both were given the same amount of fructose, the non-nephrectomized eviscerated rat would destroy more fructose in the same length of time than would the similar nephrectomized animal. This occurred in spite of the fact that the average blood fructose level was lower in the non-nephrectomized preparation.

Since it was necessary to inject glucose into the nephrectomized, eviscerated

[1] Aided by a grant from the Committee on Scientific Research of the American Medical Association.

[2] The author wishes to express his appreciation for the encouragement and inspiration given him by Dr. Leo T. Samuels.

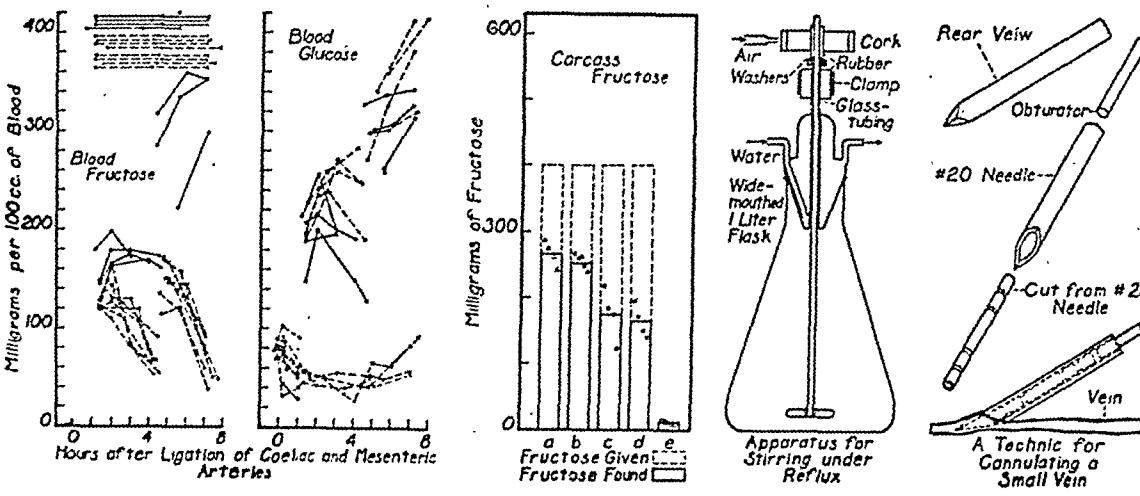

Fig. 1. The destruction of fructose by the nephrectomized and the non-nephrectomized, eviscerated rat. The rats used were approximately 200 grams in body weight. They were divided into five groups as shown in the bar graph representing the carcass fructose. (Characteristic symbols indicate individual values; heights of bars outlined in solid lines, the averages of these for the respective groups; and the height of the tops of the bars drawn in broken lines, the amount of fructose given each animal in the corresponding groups.) The animals in group "a" were allowed to fast for approximately one day. They were then eviscerated and nephrectomized; 400 mgm. of fructose in 20 grams per cent solution was given to each animal intravenously via the external jugular vein immediately at the completion of the operative procedures. After allowing 1 minute for the sugar to mix with the blood, the abdominal, thoracic and cranial cavities were opened and the carcass was plunged into enough boiling water to make a total volume of 1 liter. The water covering the carcass was kept boiling for the following 15 minutes. The carcass was then torn into small pieces with a tongs and boiled for 4 hours under reflux with stirring in the apparatus shown in the figure. A 10 cc. aliquot of the fluid portion of the resultant mixture was treated with 20 cc. of 10 per cent zinc sulfate and a drop of alcoholic phenolphthalein. Water was added to make 65 cc. and then a saturated solution of barium hydroxide until the phenolphthalein became permanently pink. The mixture was finally diluted to 100 cc. with water, and after thorough mixing a portion was filtered. A trace of concentrated sulfuric acid was added to the filtrate to neutralize the excess base. Any barium sulfate formed was removed by centrifugation and the clear supernatant fluid was analyzed for fructose (Reinecke, 1942b).

The animals in group "e" were carried through the same procedure as those in "a" with the exception that no fructose was given.

The animals in groups "b", "c" and "d" were allowed to fast about 4 days. The animals in group "b" (indicated by solid circles or solid circles connected by solid lines) were eviscerated and nephrectomized. Those in "c" (indicated by open circles or open circles connected by broken lines) and "d" (indicated by "x"s or "x"s connected by broken lines) were eviscerated but not nephrectomized. The animals in groups "b" and "c" were given fructose and glucose (two 1 cc. injections of a solution containing 20 grams per cent of each). The animals in group "d" were given fructose only (two 1 cc. injections of a solution containing 20 grams per cent). The injections were given via the saphenous veins at the time indicated by the characteristic symbols in the groups of horizontal lines at the top of the chart showing the blood fructose curves. If these lines are compared with the time scale of this chart, they show the periods during which the tissues of the individuals in each of the groups could act on the injected fructose. At the time indicated by the vertical line at the end of each horizontal line, the respective animal was chloroformed and then together with its urine, shed blood, etc., was analyzed for fructose in the manner described. Any appreciable loss of fructose was prevented by keeping each animal in a separate battery jar through-

rat to keep it alive throughout the experimental period, the effect of such injections was studied in the non-nephrectomized animal. They seem to have been without marked effect on the rate of destruction of fructose in this preparation.

The prolonged increase in the blood fructose concentration observed in the nephrectomized animal after an injection of that sugar via the saphenous vein would seem to suggest that the circulation must be very sluggish in this animal to account for such a slow mixing of the sugar with the blood. It is not possible, however, to determine from the data on the blood fructose whether or not the circulation of the non-nephrectomized animal is more adequate, for the rapid removal of the fructose from its blood would mask a similarly slow rate of mixing. The blood glucose curves, however, indicate that the sugars were mixed with the blood surprisingly slowly in both the nephrectomized and non-nephrectomized animals. This may be due to a low arterial blood pressure similar to that found by Houssay (1944) in eviscerated dogs.

The effect of fructose on the blood glucose and lactic acid of the nephrectomized and the non-nephrectomized, eviscerated rat. (See figs. 2 and 3.) Since the parenteral administration of fructose has a marked effect on the blood lactic acid (Deuel, 1936) and some effect on blood glucose (Reinecke and Samuels, 1942) in the intact animal, the effect of the injection of fructose on these constituents of blood was studied with the hope that this might give further information as to whether or not the kidney is involved in fructose metabolism.

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out the experiment and rinsing this jar with water that then was added to that in which the carcass was to be boiled. All blood samples were taken from the tip of the tail except the final one on each animal, which was taken from the right ventricle.

The individual value marked by an asterisk in group "b" on the bar graph showing the carcass fructose was not used in determining the average fructose found for its group, for the corresponding animal did not survive for quite the entire experimental period. This can be seen on inspection of the groups of horizontal lines mentioned in the preceding paragraph.

The indicated surgical procedures were completed on all the animals in groups "b"-"d" within the total elapsed time of 4¼ hours.

Numerous vanes are cut in the periphery of the cork shown at the top of the apparatus for stirring under reflux. These engage the jet of compressed air in such a fashion that it causes the cork to spin.

The drawing illustrating the technic for cannulating a small vein is modified from a figure in a thesis presented by the author to the Graduate School of the University of Minnesota in 1941 in partial fulfillment of the requirements for the Ph.D. degree in physiology. The cannula is pushed out of the needle and into the vein by the obturator. One end of a length of very slender rubber tubing ($\frac{1}{64}$ inch wall, $\frac{1}{64}$ inch bore; Davol Rubber Co., Providence, Rhode Island) is slipped over and tied to the exposed end of the cannula and then, if the cannula has been placed in an external jugular vein, the tube is threaded underneath the skin to the nape of the rat's neck where it is brought to the surface and secured with a suture. Size "A" silk is used to tie the cannula in the vein and the tube to the cannula. An injection is made by inserting a no. 27 hypodermic needle through the wall of the exposed portion of the tube. The injections of concentrated sugar solutions were routinely followed by a small injection of physiological saline to eliminate the error due to the retention of some sugar in the tube and to prevent irritation of the wall of the vein by the hypertonic solution with consequent occlusion.

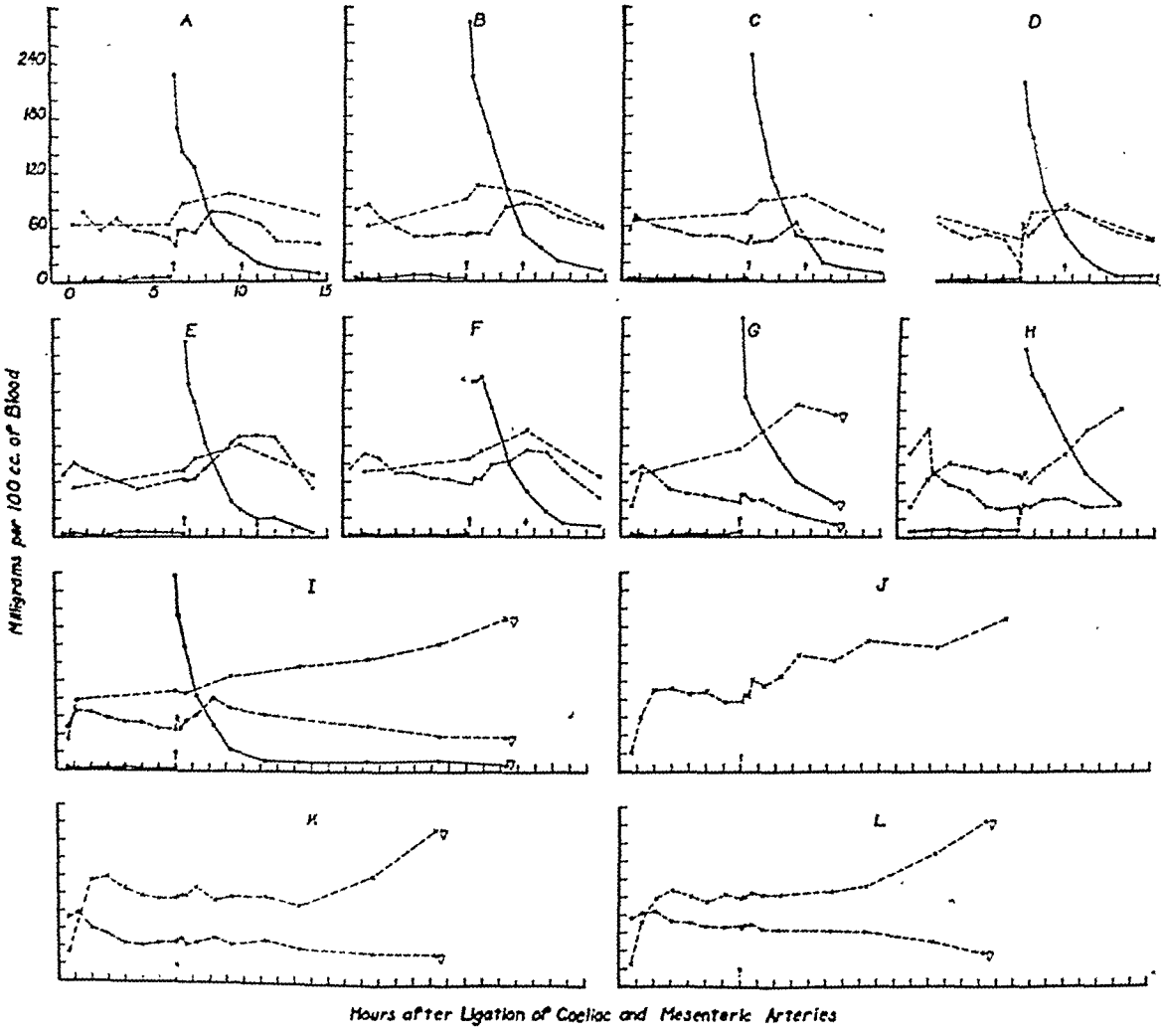

Fig. 2. The effect of fructose on the blood glucose and lactic acid of the non-nephrectomized, eviscerated rat. The animals used were approximately 300 grams in weight. Each was given a subcutaneous injection of 2 cc. of physiological saline per 100 grams of body weight just before it was anesthetized in preparation for operation. All the animals except "H" had been allowed to fast for approximately two days before being eviscerated. "H" was allowed to feed until just before evisceration. A cannula was placed in the external jugular vein of each animal just before it was eviscerated (see fig. 1). Five-tenths cubic centimeter of a 25 gram per cent solution of fructose per 100 grams of body weight (eviscerated) was given to animals "A"-"J" at the time indicated by a solid circle at the top of a solid vertical line.

"K" and "L" were given "control" injections of saline at the time indicated by a crossed circle at the top of a broken vertical line. These injections were given intravenously via the cannula in the external jugular vein. A subcutaneous injection of 0.16 unit of insulin (Iletin, Lilly) per 100 grams of body weight (eviscerated) was given to each of the animals "A"-"F" at the time indicated by the arrow. "x"s connected by broken lines indicate blood lactic acid. Open circles connected by broken lines indicate blood glucose. Solid circles connected by solid lines indicate blood fructose. All samples were taken from the tip of the tail except those marked by a small heart, which were taken from the right ventricle. "G" was given a subcutaneous injection of 2 cc. of physiological saline per 100 grams of body weight (eviscerated) immediately after the sample was taken at the ninth hour. The cannula became occluded in "F"; therefore the injection was made via the venous spaces of the glans penis in this instance.

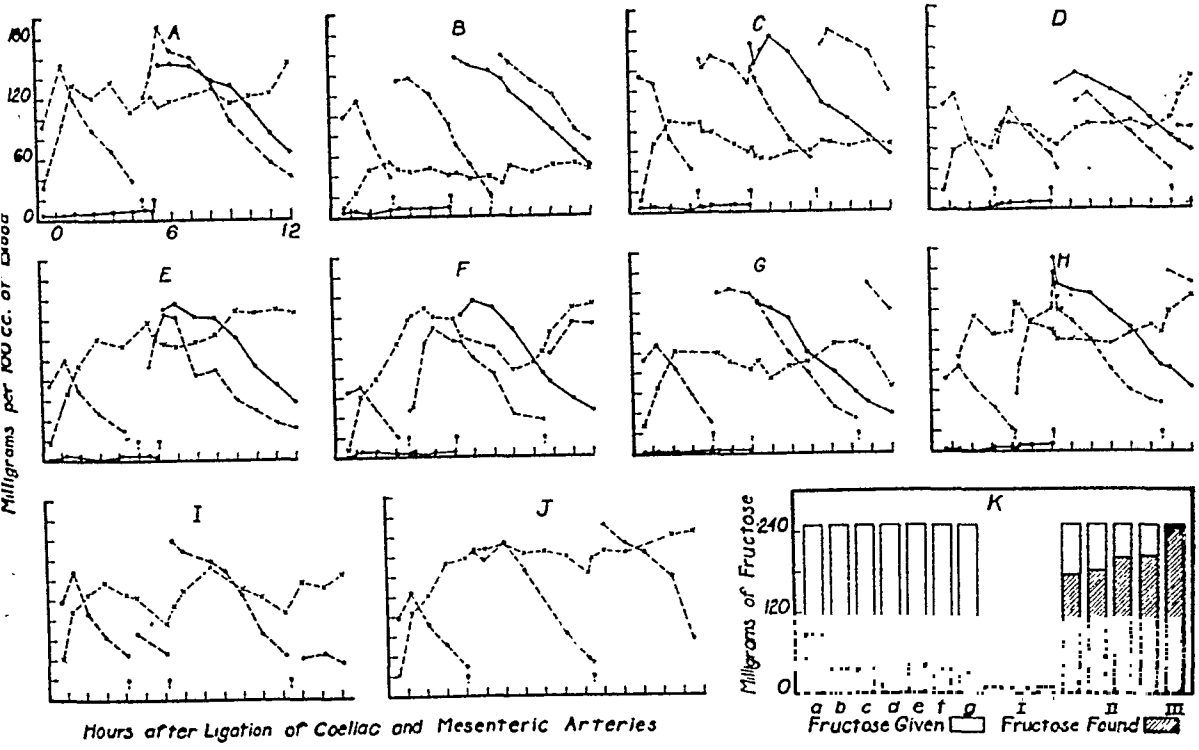

Fig. 3. The effect of fructose on the blood glucose and lactic acid in the nephrectomized, eviscerated rat. Animals of approximately 300 grams body weight were used. "A"-"D" and "I" were allowed to feed until just before they were eviscerated. "E"-"H" and "J" were allowed to fast for from 4 to 5 days before being operated upon. Each animal was given a subcutaneous injection of 2 cc. of physiological saline per 100 grams of body weight just before being anesthetized for operation. A cannula was placed in an external jugular vein (see fig. 1) just before the evisceration proper which in turn was followed by nephrectomy. One cubic centimeter injections of a 25 gram per cent fructose solution were given at the times indicated by solid circles at the tops of solid verticle lines. One cubic centimeter injections of a 25 gram per cent glucose solution were given at the times indicated by open circles at the tops of vertical broken lines. All of these injections were made through the cannula except in "I" where the first injection made through the cannula was unsatisfactory; a second injection via the venous spaces of the glans penis proved more satisfactory; a third injection also via the penis, however, failed. "x"s connected by broken lines indicate blood lactic acid. Open circles connected by broken lines indicate blood glucose. Solid circles connected by solid lines indicate blood fructose. All samples were taken from the tip of the tail. Immediately after the last sample shown in their respective curves was taken, animals "A"-"H" were analyzed for carcass fructose. Each rat was anesthetized with intravenous sodium amytal and then passed through a small meat grinder three times. The resultant finely chopped tissue was quantitatively transferred to the apparatus for stirring under reflux by several washes of distilled water. Enough water was then added to make about 1 liter and the whole was boiled with stirring under reflux for 15 minutes. The mixture was then filtered through muslin and the solids were returned to the apparatus. Enough water was added to make about 1 liter and the whole was again boiled with stirring under reflux for another 15 minutes. The mixture was filtered through muslin as before and the solids again returned to the apparatus with enough water to make 1 liter, but 30 cc. of concentrated sulfuric acid were also added. This time the boiling with stirring under reflux was continued for 30 minutes. This acid digest was then added to the previous filtrates to make a total volume of about 3 liters; 30 cc. of this was treated with a drop of alcoholic phenolphthalein and 20 cc. of 10 per cent zinc sulfate. Enough of a saturated solution of barium hydroxide was then added to give a permanent pink. The mixture

The mixing of the injected fructose with the blood was much improved by making the injections via a cannula tied in an external jugular vein.

In all of the ten non-nephrectomized rats given fructose there was a definite increase in blood lactic acid following the injection of that sugar. In two similar animals given control injections of physiological saline there was no comparable increase. The concentration of lactic acid in the blood of the nephrectomized, eviscerated rats was much more variable, but there was no consistent increase following the administration of fructose.

Six of the ten non-nephrectomized, eviscerated rats given fructose also exhibited a definite elevation in their blood glucose concentrations following the injection. In one (C) of the other four there was a small, rather indefinite increase. Another of these (G) showed a rather short survival period, which is characteristic of a poor preparation. The third animal (H) had been fed until just before evisceration, which in itself is sufficient to reduce the ability of the kidney to maintain the blood sugar level (Reinecke and Roberts, 1944) and which hence may also possibly adversely affect the renal function concerned with fructose metabolism. In a fourth animal (J) the blood glucose was not observed. No definite effect of fructose on the blood glucose of the nephrectomized, eviscerated animals was found. But the interpretation of the data is complicated in this instance by the necessity for repeated injections of glucose to maintain the animals alive.

Because the analyses of the carcasses of the nephrectomized animals in the first experiment (fig. 1) did not indicate a significant destruction of fructose in contrast to the findings of a previous study (Reinecke, 1942a) the carcasses of the nephrectomized animals used in this experiment were also analyzed for fructose. The destruction of fructose by the nephrectomized, eviscerated rat as reported before was confirmed. The apparent failure of the nephrectomized animals used in the first experiment to destroy fructose seems to have been due to the fact that the conditions chosen for the experiment—comparatively large doses of fructose with a comparatively short period allowed for the tissues to act—would tend to mask a slow destruction of the sugar.

The non-nephrectomized animals given no fructose showed a marked increase

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was diluted to 100 cc. and stirred. A portion was filtered and the filtrate was acidified with a trace of concentrated sulfuric acid. Any barium sulfate formed was removed by centrifugation. Fructose was determined in the clear supernatant fluid (Reinecke, 1942b). The results are shown in graph "K". The lettered bars are for respectively the same animals as the individual graphs of blood constituents with corresponding letters. Unfortunately the carcass analysis of "H" was spoiled. The bars in group I indicate the values obtained from the analysis of similar animals that had been given no fructose. Those in group II indicate the values obtained from the analysis of animals given fructose and then killed after only a moment had been allowed for the fructose to be distributed throughout the tissues. The bar marked III shows the value obtained by analysis of a sample from 3 liters of water to which fructose and 30 cc. of concentrated sulfuric acid had been added. The precipitation of zinc hydroxide and barium sulfate was carried out in the described manner on this sample.

The initial portions of the glucose curves of "A"-"J" were presented in another paper (Reinecke and Roberts, 1944).

in blood lactic acid during the latter part of their period of survival. It is interesting to note that the only definite decreases in blood lactic acid levels in non-nephrectomized animals occurred after the administration of small doses of insulin. The explanation for this is not at present apparent.

Discussion. The findings that fructose disappears more rapidly in the presence of the kidney, and that injections of fructose will cause an elevation of blood lactic acid and glucose levels in the eviscerated rat that retains its kidneys, but that the lactic acid level is not affected in the animal from which the kidneys have been removed, all are strongly suggestive that the kidneys are organs of particular importance in the metabolism of fructose. The finding of Corkill and Nelson (1939) that the infusion of fructose was without effect on the blood lactic acid levels of the spinal, eviscerated cat is supported, for these investigators seem to have removed the kidneys from their preparation. However the results of Sterkin et al. (1939) that the injection of fructose was without effect on the blood lactic acid and glucose of the hepatectomized dog and those of Bollman and Mann (1931) that it was without influence on the blood glucose level of the non-nephrectomized, eviscerated dogs are at variance with the present findings. The basis of the discrepancy may be a species difference; but it seems to the author that it is also possible that matters of technic are involved, for it has been found that another and probably related function, namely, the maintenance of the blood sugar level in the eviscerated rat, is suppressed by the loss of small amounts of blood during and after evisceration (Reinecke, 1943). The evisceration of the rat is technically a simple procedure while the evisceration of the dog is a relatively formidable operation. It must also be recalled that the method used for estimating the blood glucose in these experiments is such that some non-fermentable reducing substances appear as glucose.

The terminal elevation in the blood lactic acid level in the non-nephrectomized, eviscerated rat that had been given no fructose supports a similar finding by Drury and McMaster (1929) in the hepatectomized rabbit.

SUMMARY

Non-nephrectomized, eviscerated rats were found to destroy fructose more rapidly than similar nephrectomized animals.

The injection of fructose was found to cause an elevation in the blood glucose and lactic acid levels in the non-nephrectomized, eviscerated rat. Similar changes in the concentrations of these substances were not observed in the blood of the nephrectomized, eviscerated rat.

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THE EFFECT OF BLOOD WITHDRAWAL AND REPLACEMENT ON THE BLEEDING VOLUME OF NORMAL DOGS UNDER BARBITAL ANESTHESIA[1]

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Received for publication March 18, 1944

In recent studies on blood substitutes the volume of blood which must be withdrawn to produce circulatory standstill has been used for comparing the adequacy of circulating fluids (Lawson, 1943; Lawson and Rehm, 1943). The studies were made possible by the observation that a fairly reliable control estimate of the total bleeding volume may be obtained by extrapolation from the data on mean arterial pressure after partial exsanguination. The animals survive for several hours if the blood drawn for the control estimation is replaced, and can be bled to death subsequently for measurement of the change in bleeding volume. The chief consideration in the early studies was the selection of experimental conditions under which the amount of change in bleeding volume after the replacement would depend upon the composition of the fluid used. The experimental conditions were for this reason highly specialized, and included a rather severe conditioning and screening hemorrhage several hours before the control estimation of bleeding volume. These conditions are obviously not ideal for more fundamental studies.

The volume of blood which must be withdrawn in order to produce permanent stoppage of arterial flow is

$$H = Q + I - L,$$

where H is the volume drawn (bleeding volume), Q is the initial blood volume, I the volume exchanged with extravascular spaces during the bleeding, and L the liminal volume of blood required to maintain a finite cardiac output. Consideration of the hemic and hemodynamic factors which could influence the two latter values suggests that bleeding volume may reflect a variety of circulatory changes. Its possible utility as a measurable quantity warrants investigation of the conditions under which it may be measured in normal animals.

The present report examines the reliability of the extrapolation for the residual bleeding volume after partial exsanguination in normal dogs, without special preparation or selection. An attempt is made to find conditions under which bleeding volume does not decline following partial exsanguination and replacement. Indirect evidence is presented that bleeding volume is a linear function of blood volume within certain limits.

METHODS. Unselected dogs, kept without food and water for 24 hours, were injected intravenously with 250 mgm./kgm. sodium barbital after anesthesia had been induced with ether. Mean arterial pressure was recorded from a mer-

[1] Aided by a grant from the Knox Gelatin Company, Johnstown, N. Y.

cury manometer connected with a femoral artery, the contralateral femoral artery being cannulated for bleeding, and a femoral vein cannulated for infusions. The animals were tracheotomized and kept in a supine position throughout the period of observation with non-obstructing leg holders.

Blood was drawn by free hemorrhage through the femoral bleeding cannula in unit volumes of 2 cc./kgm., the withdrawals being started at intervals of 2 minutes. Pressure was read at the end of each interval, as in the former studies. When total bleeding volume was to be measured, the interval bleedings were discontinued as soon as mean arterial pressure fell below 10 mm. Hg, a fractional withdrawal on the last bleeding being recorded as either 0 or 2 cc./kgm., whichever was the nearer.

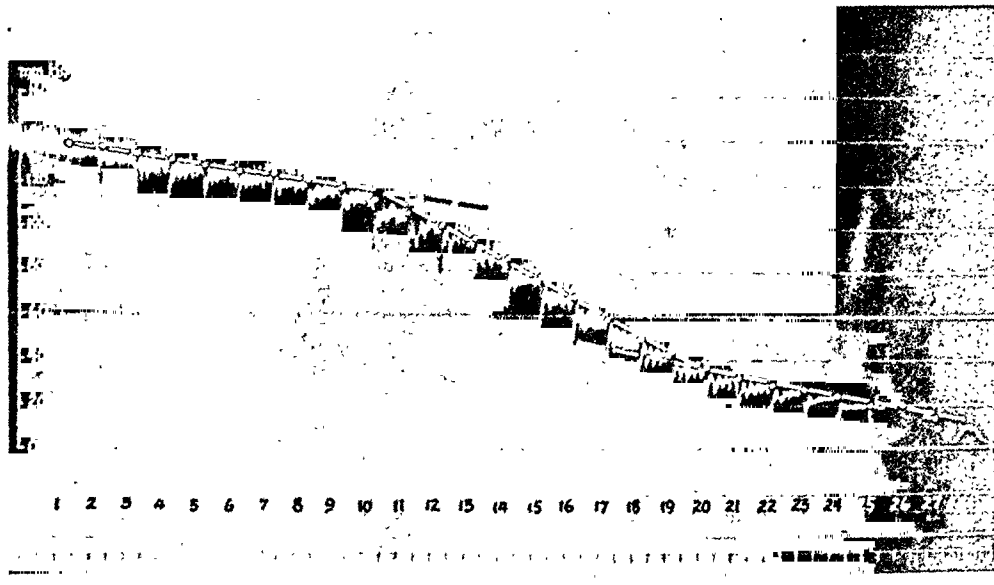

Fig. 1. *Mean arterial pressure during controlled hemorrhage at the rate of 2 cc./kgm./2 min.* Duration barbital anesthesia 1¾ hours at start of tracing. Each numbered signal at the bottom marks withdrawal of 2 cc. blood per kgm. body weight by free arterial bleeding. Pressure readings, taken just before each withdrawal are given as points, which have been roughly fitted to 3 straight lines. Time recorded in minutes. The height of pressure at the start of the tracing is not typical.

In the replacement experiments autogenous blood was used, heparinized as it was drawn during the control estimation. Replacement was started 2 minutes after the last interval bleeding, and was completed within 3 to 8 minutes.

Results. *The effect of bleeding on mean arterial pressure.* Figures 1 and 2 illustrate the two types of records obtained under these conditions when the bleeding was continued to stoppage of the circulation. Qualitatively the records resemble those obtained in the previous studies on pre-hemorrhaged animals. The initial and terminal linear segments, along which the rate of pressure decline remains constant, correspond to the initial and terminal pressure plateaus of the former reports. The middle segment in the records was usually also linear during the first 2 to 3 hours of anesthesia, with sharp points of inflection above and be-

low, as in figure 1. After the third hour, linear middle segments were rarely observed (fig. 2). Records qualitatively resembling figure 1 have been obtained in animals subjected to this type of bleeding under ether anesthesia, the depth of anesthesia being reduced as pressure fell.

The partition of bleeding volume at 60 mm. Hg. Extrapolation for the residual volume after partial exsanguination was based in the former studies on the finding that bleeding volume, under the conditions of the studies, was divisible into a variable volume obtained above 60 mm. Hg mean arterial pressure, and a relatively constant volume obtained after pressure had fallen below 60 mm. The control estimate of bleeding volume could thus be made simply by measuring the variable volume required to lower pressure to 60 mm., and adding the fairly constant residual volume found to be obtainable below that level in control animals.

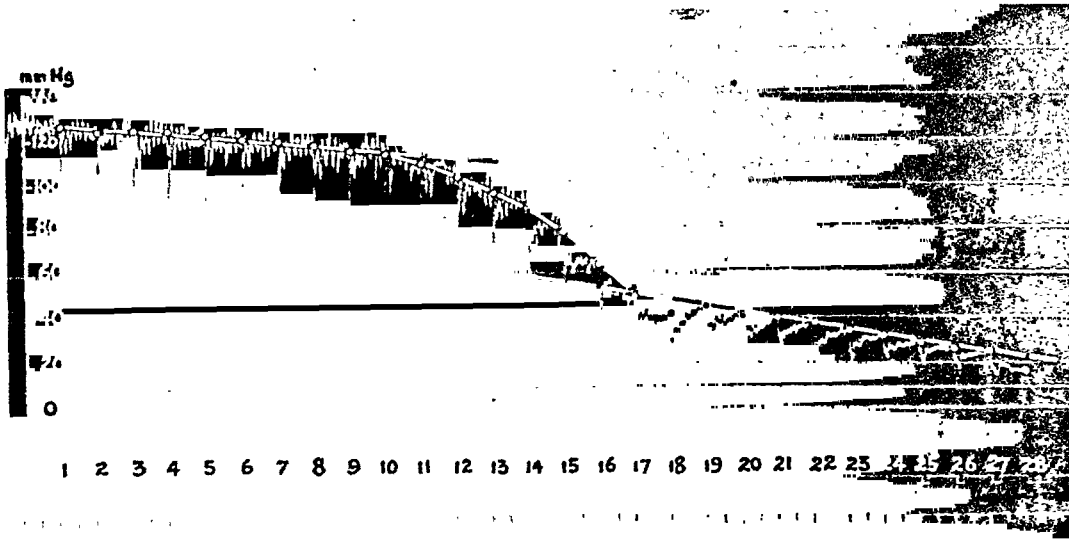

Fig. 2. Construction of figure as in figure 1. Duration barbital anesthesia at start of tracing 3½ hours. The fall in pressure below the terminal linear segment at the 18th reading is not typical.

Division of the total bleeding volume into corresponding fractions has been done in figure 3 for a group of normal animals bled to death under the present conditions. The average volume drawn above 60 mm. was 25.6 cc./kgm., s.d. = 7.76 cc./kgm.; the average volume drawn below 60 mm. was 22.5 cc./kgm., s.d. = 3.24 cc./kgm. The coefficient of variation for the upper fraction is thus 30.3, while for the lower fraction it is only 14.4. Since the lowest coefficient of variation for the lower fraction observed with any rate of bleeding in the former studies was 19.3, it is apparent that the special conditions of the former studies are not responsible for the relative constancy of the volume obtainable below 60 mm. The limited variations in the lower voume do not appear from the data of figure 3 to be related in any systematic fashion to variations in the volume drawn at the higher pressure levels.

The volumes drawn below 60 mm. Hg in this group of animals are plotted in

figure 4 against the duration of barbital anesthesia. There is no evidence in the data for any progressive change in this volume during the first 10 hours of anesthesia.

If bleeding is terminated at 60 mm. Hg under these conditions, the residual bleeding volume may be expected, at a probability of 0.05, to lie between 16 and 28 cc./kgm., with an average value in the neighborhood of 22.5 cc./kgm. The expected limits of variation are corrected, for the purposes of this prediction, to the nearest multiple of 2 because of the treatment of fractional bleedings described above. In obtaining these data an attempt has been made to include animals with extreme variations in the volume obtainable above 60 mm., and the

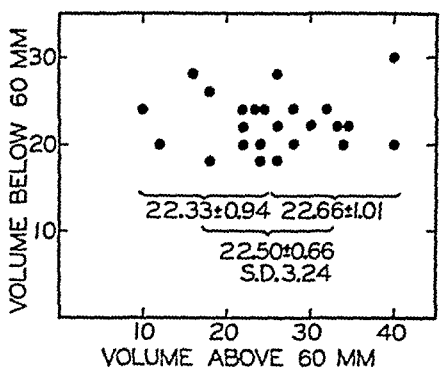

Fig. 3

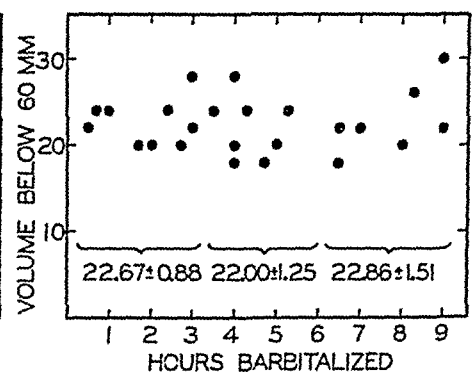

Fig. 4

Fig. 3. *Relationship between the volumes drawn above and below 60 mm. Hg mean arterial pressure during complete exsanguination at the rate of 2 cc./kgm./2 min.* Each point represents a single animal. The bracketed figures at the bottom are the means, with their standard errors, of the volumes obtained below 60 mm. when the volumes drawn at the higher levels were above and below the median, respectively. The lowest bracket gives the mean of all the volumes drawn below 60 mm., with s.e. and s.d. All volumes as cubic centimeters per kilogram.

Fig. 4. *The effect of prolonged barbital anesthesia on the volume obtainable below 60 mm. Hg.* Same group of animals as in figure 3. The volumes drawn after mean arterial pressure had fallen below 60 mm. are plotted against the duration of anesthesia at the start of the bleeding. Volumes as cubic centimeters per kilogram. The bracketed figures at the bottom give the mean volumes obtained, with their standard errors, during the first, second and third 3-hour periods.

series has been extended somewhat beyond the period of anesthesia expected to suffice for most experiments. It is probable that the limits of variation in the residual volume at 60 mm. have been liberally estimated.

The change in total bleeding volume under barbital anesthesia. At the start of these studies it was customary to barbitalize at the beginning of the day all the animals to be used, and to leave them undisturbed until it was convenient to work on them. After a sufficiently large number of animals had been bled to the 60 mm. end-point it became apparent that the volume of blood drawn above 60 mm. depended upon the duration of anesthesia. These data are summarized in table 1, from which it can be seen that this volume is at a maximum during the fourth hour. The increase during the first 3 hours is accompanied by a rise in arterial

TABLE 1

The effect of prolonged barbital anesthesia on bleeding volume and on mean arterial pressure

The third column gives mean arterial pressure at the beginning of the bleeding. The fourth column gives the volume which had to be drawn at the rate of 2 cc./kgm./2 min. to lower pressure to 60 mm. Hg. Total bleeding volume may be estimated by adding 22.5 cc./kgm. to these values. The fifth column gives the volume obtained on the first linear segment of the pressure records. Values given are means with their standard errors.

HOURS BARB.	NO. DOGS	ART. PRESSURE	VOL. ABOVE 60 MM.	VOL. 1ST LIN. SEG.
		mm. Hg	*cc./kgm.*	*cc./kgm.*
1st	6	110.0 ±6.448	22.3 ±4.143	14.0 ±4.261
2nd	21	112.2 ±3.318	25.1 ±1.365	13.5 ±1.492
3rd	37	120.7 ±2.618	27.1 ±1.243	15.1 ±1.500
4th	42	123.5 ±2.470	30.2 ±1.045	20.2 ±0.883
5th	21	125.5 ±3.288	27.8 ±1.258	20.1 ±1.098
6th–10th	26	124.2 ±2.286	24.3 ±1.263	17.6 ±0.923

TABLE 2

Bleeding volume after withdrawal of the volume above 60 mm. and injection of uniform volumes of blood

The volumes drawn in lowering pressure to 60 mm. Hg are given under the heading H_{1a}. Total bleeding volumes at this time are estimated by adding 22.5 cc./kgm. to these values, and the estimates are given under the heading H_1. The volume of blood re-injected is given under R. Only autogenous blood was used, except for the case marked with an asterisk, in which 6 cc./kgm. were obtained from a donor animal. The potential bleeding volume after bleeding and replacement is estimated as the sum of the residual and the replacement volumes, and is given under the heading $R + H_{1e}$. The volumes obtained on complete exsanguination after an interval of approximately 4 hours are given under the heading H_2.

DOG NO.	H_{1a}	H_1 (ESTIMATE)	R	$R + H_{1e}$ (ESTIMATE)	H_2
1	36	58.5	10		30
2	28	50.5	10		32
3	24	46.5	10		30
4	32	54.5	10		34
5	28	50.5	10		32
Mean		52.1		32.5	31.6
S. D.		4.58		3.24	1.67
6	30	52.5	20		44
7	26	48.5	20		42
8	34	56.5	20		40
9	40	62.5	20		38
10	24	46.5	20		44
11	14	36.5	20*		44
Mean		50.5		42.5	42.0
S. D.		8.94		3.24	2.53

pressure and an increase in the length of the initial linear segment in the pressure records. The decline after the fourth hour is not accompanied by a fall in arterial pressure, although the initial linear segment in the records appears to be somewhat shortened.

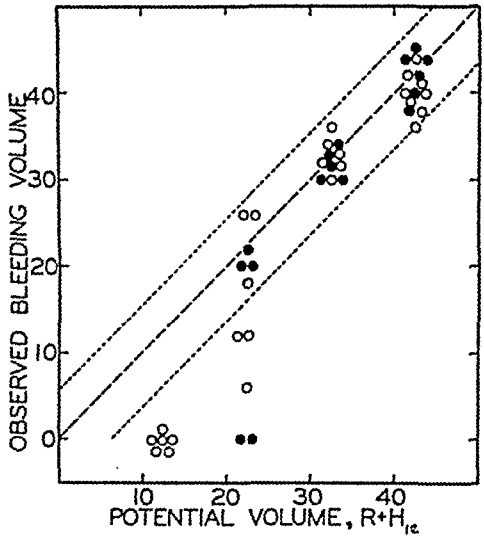

Fig. 5

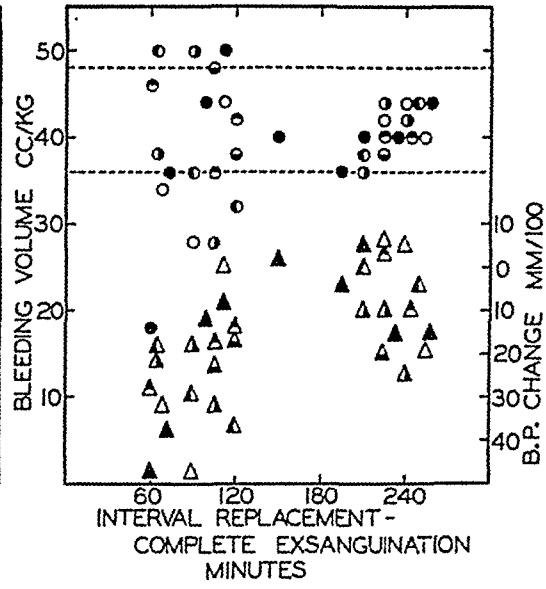

Fig. 6

Fig. 5. *Relationship between observed and expected bleeding volumes.* The broken 45° line gives the mean volumes expected if bleeding volume after withdrawal and replacement of blood is equal to the sum of the residual and the replacement volumes ($H_{1e} + R$). The parallel dotted lines give the expected limits of variation at 0.05 probability due to the error in estimating the residual volume (H_{1e}). The partial exsanguination was terminated at 60 mm. Hg in the case of the solid circles, and the value estimated for H_{1e} is 22.5 cc./kgm. An additional 10 cc./kgm. were withdrawn before replacement in the case of the hollow circles, and H_{1e} was estimated as 12.5 cc./kgm. The volume of replacement (R) may be obtained by subtracting from the abscissae 22.5 for the solid circles, and 12.5 for the hollow circles. Interval between replacement and the final measurement of bleeding volume approximately 4 hours. All volumes as cc./kgm.

Fig. 6. *The change in bleeding volume and mean arterial pressure immediately following bleeding and replacement.* Expected bleeding volume ($H_{1e} + R$) = 42.5 cc./kgm. average. In all cases where the interval was less than 180 minutes, the partial exsanguination was terminated at 60 mm. Hg, R was 20 cc./kgm., and H_{1e} was estimated as 22.5 cc./kgm. Bleeding volumes are given as circles, the percentage change in mean arterial pressure at the start of the bleeding as triangles, pressure at the start of the initial bleeding being taken as 100 per cent. Similarity of shading within the symbols permits identification of blood pressure with bleeding volume, where more than one animal lies on the same ordinate. The dotted lines near the top of the figure give the limits of variation in bleeding volume expected at 0.05 probability from the error in estimating H_{1e}.

The bleeding was terminated at 60 mm. in most of these animals. Since, however, the data of figure 4 show that the volume obtainable below 60 mm. does not change with the duration of anesthesia, it is assumed that the average residual volume for all the groups in table 1 is approximately 22.5 cc./kgm., and the data are taken to represent changes in total bleeding volume. It appears from these

lata that total bleeding volume may decline as much as 6 cc./kgm. in undisturbed animals between the fourth and the tenth hour of barbital anesthesia.

Bleeding volume after withdrawal of the variable fraction and injection of uniform volumes of blood. If animals are bled to a uniform residual bleeding volume and injected with equal volumes of blood, they may be expected to yield equal total bleeding volumes on subsequent exsanguination only if the volume of blood is the principal limiting factor for bleeding volume. This possibility was examined in experiments which are summarized in table 2 and figure 5. In these and subsequent experiments of this report the animals were left undisturbed until after the third hour of anesthesia. Widely scattered data were frequently obtained when bleeding and replacement were done during the first 3 hours, the volumes measured subsequently being as a rule considerably below their expected values. In both the table and the figure the interval between the partial exsanguination and replacement and the measurement of total bleeding volume was approximately 4 hours. Detailed data are given in the table for two groups to show that uniformity of total bleeding volume under these conditions can be obtained in animals whose control bleeding volumes are widely different. The data for all the experiments are summarized in the figure. In about half the experiments an additional 10 cc./kgm. were withdrawn after pressure had fallen to 60 mm. As is shown in the figure, such reduction in the residual volume does not seem to influence total bleeding volume if the replacement volume is correspondingly increased.

Total bleeding volume appears from these data to be almost exactly equal to the potential bleeding volume estimated as the sum of the residual and the replacement volumes, so long as the potential volume is in excess of some value between 22.5 and 32.5 cc./kgm. The small deficits observed when the potential volume was above this minimal value could be attributed entirely to the decline in bleeding volume under barbital.

The time required for stabilization of bleeding volume after blood withdrawal and replacement. In figure 6 the values obtained for total bleeding volume are plotted against the duration of the interval between the partial bleeding and replacement and the measurement of total volume. The figure gives data for animals with estimated potential volumes of 42.5 cc./kgm. The figure shows that bleeding volume may deviate in either direction beyond its expected limits of variation for at least $2\frac{1}{2}$ hours after blood withdrawal and replacement. The mean of the observations at intervals less than $3\frac{1}{2}$ hours is not significantly different from the mean of later observations. But the ratio of the variances is 10.74:1, which has a probability considerably less than 0.01. The data of the figure are therefore interpreted as showing that bleeding volume may be significantly increased or decreased for as long as $2\frac{1}{2}$ hours after a partial bleeding and replacement. The data given in the figure on mean arterial pressure show that pressure usually recovers slowly following the replacement, and that the recovery is far from complete during the unstable early period.

The partition of bleeding volume at 60 mm. Hg after bleeding and replacement. The data of figure 7 show that the partition of the total volume into a variable

volume obtainable above and a constant volume obtainable below 60 mm. Hg, is considerably disturbed for some time following partial exsanguination and replacement. The figure shows the expected values for both fractions in the foregoing experiments, in comparison with the values actually observed. In general, the fraction obtained above 60 mm. appears to have been increased at the

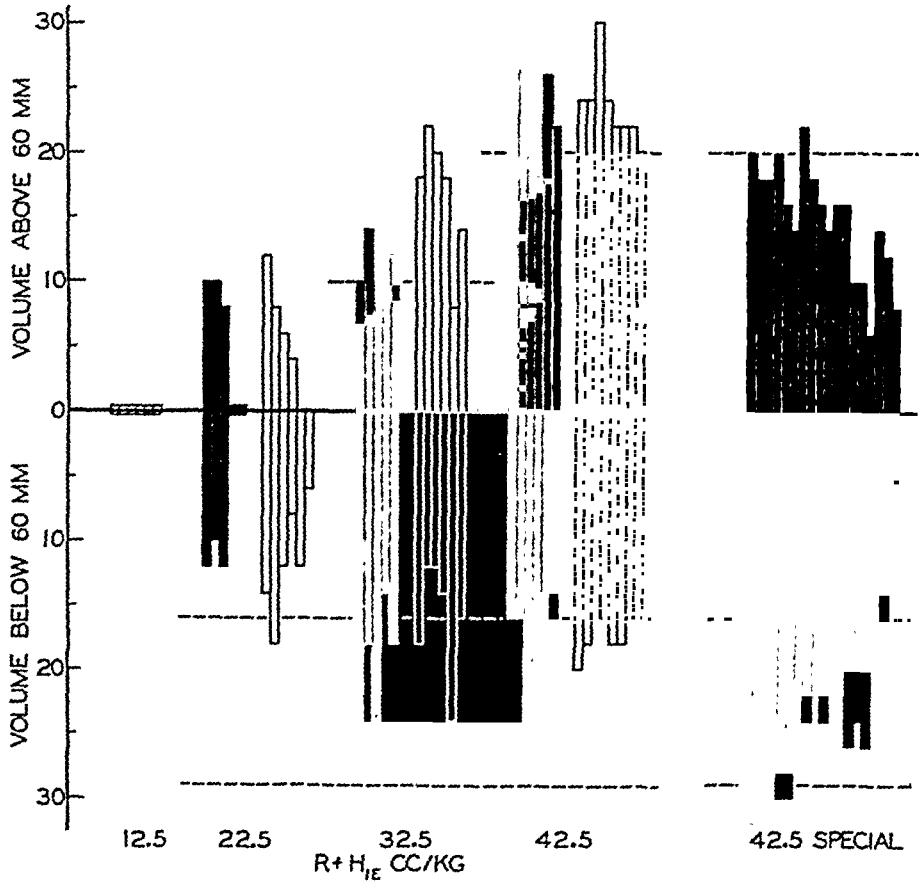

Fig. 7. *The partition of total bleeding volume at 60 mm. after bleeding and replacement.* Potential total bleeding volume is given below each block of data as $R + H_{1e}$, where R is the volume of blood reinjected, and H_{1e} is the estimated residual volume at the end of the control bleeding. $H_{1e} = 22.5$ cc./kgm. for the cases shown in black, 12.5 cc./kgm. for the cases shown in white. The broken lines in the lower half of the figure mark the limits of variation at 0.05 probability in the volume obtainable below 60 mm. from animals without previous bleeding and replacement. The broken lines in the upper half of the figure give the volumes expected above 60 mm., where $(R + H_{1e}) - 22.5$ is greater than 0. Interval between control and final observation approximately 4 hours except for group marked "special." In the latter the interval was 1–2½ hours. In each block the data are arranged in order of diminishing total bleeding volume. All volumes as cubic centimeters per kilogram.

expense of the fraction obtained below. The shift of volume from the lower to the upper fraction seems to take place slowly, as there is no suggestion of it earlier than 2½ hours after the bleeding and replacement. It does not appear to be closely related to the net volume of blood lost in the bleeding and replacement, since it was not diminished by increasing the volume of replacement.

Discussion. These data show that a fairly reliable estimate of total bleeding volume may be made by partial exsanguination in normal dogs under barbital anesthesia without sacrificing the animals. The error in the estimate, at 0.05 probability, is not greater than 6 cc./kgm. The percentage error at this probability lies between 9 per cent for the larger and 16 per cent for the smaller total bleeding volumes observed after the third hour of anesthesia, when the partial exsanguination is terminated at 60 mm. Hg mean arterial pressure; and between 8 per cent and 13 per cent if an additional 10 cc./kgm. are drawn. Since both the absolute and the percentage error are somewhat less than in the former studies it is obvious that special preparation and selection of animals are not essential. No animals were excluded from the data of the present report except for an occasional case in which gross irregularities were obtained in the pressure data as the 60 mm. end-point for the partial exsanguination was approached.

The volume which can be withdrawn below 60 mm. in normal dogs is larger and less variable than the volume which could be withdrawn, at the same rate of bleeding (2 cc./kgm./2 min.), in the former studies. That the difference is due to the preparatory hemorrhage of the former studies is suggested by controlled data, in the present report, on the re-partition of total bleeding volume after blood loss.

It appears likely that bleeding volume measurements will be more useful in the detection of circulatory changes than in their elucidation. The multiplicity of the factors which may influence bleeding volume is expected to contribute to the sensitivity of bleeding volume as an index to certain obscure circulatory changes, but at the same time makes interpretation highly speculative without additional data.

It is well known that complex changes occur in the composition of blood and in hemodynamics during the first few hours of barbiturate anesthesia (Adolph, Gerbasi and Lepore, 1933; Adolph and Gerbasi, 1933; Jarcho, 1943; Green, Nickerson, Lewis and Brofman, 1943). It is not surprising, therefore, that bleeding volume also changes. The small bleeding volumes of the first 3 hours are associated with arterial pressures considerably lower than those reported for unanesthetized dogs by Corcoran and Page (1943). With this supporting evidence, they are tentatively interpreted as temporarily depressed volumes, with recovery reaching a maximum some time between the fourth and sixth hours, along with arterial pressure. The terminal decline in bleeding volume under barbital is unaccompanied by a change in arterial pressure, which remains after the third hour at the levels reported for unanesthetized dogs. It probably does not represent, therefore, simply a reversal of the mechanisms responsible for the initial increase in volume. This conclusion is supported by the observation that bleeding and replacement during the first 3 hours, but not during the terminal period, may be followed by a rapid decline in both arterial pressure and bleeding volume.

The graded replacement experiments were designed primarily for the purpose of examining the change in the residual bleeding volume with time at various levels of exsanguination. The residual bleeding volume at any time t during a complete exsanguination, is

$$H_t = Q_t + I_{t-d} - L_d,$$

where H_t is the volume remaining undrawn, Q_t is the blood volume at that instant, I_{t-d} is the volume which will be gained or lost in exchange with extravascular spaces between time t and stoppage of the circulation, and L_d is the liminal blood volume which will be required to maintain arterial flow when the exsanguination nears completion. The value of both I and L might be expected to change with time if a complete exsanguination were interrupted for several hours during its course. Unless equal changes occurred in I and L, the volume obtained on resuming the bleeding would not be expected to equal H_t. The amount of change in I and L, and the resulting deviation from the expected bleeding volume, may be expected to depend upon the degree of exsanguination at the time of the interruption, as well as upon the duration of the interruption.

In order to obtain the estimate of H_t required for the study, it was necessary in the experiments to withdraw completely the variable volume obtainable above 60 mm., since extrapolation for the residual volume at higher pressure levels does not seem to be feasible. The residual volume was then adjusted to various levels of net blood loss by replacements or further withdrawals. The data obtained fail to reveal any difference in total bleeding volume at the end of 4 hours, between animals subjected to over-bleeding and replacement, and those subjected to an equal net blood loss without over-bleeding. The data on graded replacement may therefore be regarded as valid for the purposes of the study, and $(H_{1e} + R)$ in the experiments may be substituted for H_t in the equation above. H_{1e} is the estimated residual volume at the end of the partial exsanguination, and R is the volume of blood re-injected.

In the experiments in which bleeding volume at the end of the interruption was found to be equal to H_t $(= H_{1e} + R)$, it is apparent that any growth in I_{t-d} during the interval must have been matched by an equivalent increase in L_d. It is further apparent that this relationship does not depend upon the level of exsanguination, so long as H_t remains above a certain critical level. It is well known that fairly rapid regeneration of plasma occurs after non-critical losses of blood, so that volume losses of 25 to 40 cc./kgm. may be completely recovered within 12 to 24 hours (Hooper, Smith, Belt and Whipple, 1920; Ebert, Stead and Gibson, 1941; Ebert, Stead, Warren and Watts, 1942). It seems unlikely, therefore, that the value of I in the equation above remains fixed for 4 hours in the present experiments. In the more likely event that it grows with the increase in the interval, the present findings must mean either that the plasma volume added during the 4 hour interval is completely lost to the tissues when the bleeding is resumed, or that compensating changes occur in the value of L_d during the interval.

Any deviation in bleeding volume from its expected value in these experiments must be due to uncompensated changes in I or L in the equation. Since significant deviations were observed for at least 2 hours following bleeding and replacement, it is obvious that at least one of these factors must continue to change for a time. The disappearance of the deviations at the end of 4 hours could mean

either complete reversal, or the achievement of compensatory growth in the remaining factor. The re-partition of bleeding volume at 60 mm. even after nearly complete replacement suggests that 4 hours are not long enough for reversal of all the changes, and thus favors the latter interpretation. Additional data on this point are obviously needed.

The data offer evidence that bleeding volume is a linear function of the volume of blood possessed by the animal, within a limited range. Individual variations in bleeding volume were made to disappear by bleeding the animals to a uniform residual bleeding volume, and injecting equal volumes of blood. Increasing the volume injected produced an equal increase in bleeding volume. Control measurements of bleeding volume show individual variations of the same order as those reported for blood volume in unselected dogs by Bonnycastle and Cleghorn (1942). The present data suggest, therefore, that variations in blood volume are largely responsible for the variations in bleeding volume observed on the initial exsanguination.

SUMMARY AND CONCLUSIONS

The residual bleeding volume can be estimated in normal unselected dogs under barbital anesthesia by extrapolation from the data on mean arterial pressure after pressure has been lowered to 60 mm. Hg by controlled hemorrhage. The error in the estimation, at 0.05 probability, is not greater than 6 cc./kgm., or 8 per cent to 16 per cent of the total bleeding volume. The absolute error in the extrapolation does not appear to change with the duration of anesthesia up to 10 hours, nor to vary with the total bleeding volume. Both bleeding volume and mean arterial pressure rise during the first 3 hours of barbital anesthesia. Bleeding volume, but not arterial pressure, declines between the fifth and tenth hours of anesthesia.

When animals were bled under these conditions until the residual bleeding volume as estimated by extrapolation was the same in all cases, and were reinjected with equal volumes of blood, they were found to have nearly uniform total bleeding volumes 4 hours later. If the potential bleeding volume was estimated as the sum of the residual and the replacement volume, the volume obtained was found to be approximately equal to the potential volume, so long as the latter exceeded a critical value lying between 22.5 and 32.5 cc./kgm. When bleeding volume was measured earlier than $2\frac{1}{2}$ hours after the initial bleeding and replacement, the volumes obtained deviated in both directions beyond limits permitted by the error in estimating the potential volume. The partition of total bleeding volume into a variable volume obtainable above, and a relatively constant volume obtainable below 60 mm. Hg mean arterial pressure, is considerably disturbed for more than 4 hours after partial exsanguination and replacement.

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THE EFFECTS OF EXCITEMENT, OF EPINEPHRINE AND OF SYMPATHECTOMY ON THE MITOTIC ACTIVITY OF THE CORNEAL EPITHELIUM IN RATS[1]

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Received for publication March 20, 1944

In a previous report (4) methods for testing the mitotic activity in the corneal epithelium of rats have been described. Two substances, cocaine and ephedrin, which have been shown with these methods to inhibit mitotic activity, are known to sensitize various effector organs to the effects of epinephrine. A third substance, ether, which was found to inhibit mitotic activity, is known to stimulate the secretion of epinephrine by the adrenal glands. It was natural, therefore, to inquire whether epinephrine itself would alter the mitotic activity of the rat's corneal epithelium.

Our interest in the possible influence of epinephrine was stimulated also by the following observations. When rats were taken from cages in which they had previously been undisturbed, and immediately sacrificed, mitosis counts on their corneas were found to be at what we have previously reported to be the normal level. If, however, rats were transferred from one cage to another, or subjected to various control procedures such as the intramuscular injection of salt solution or the instillation of salt solution into one eye, or annoyed in some other manner and then sacrificed for mitosis counts about an hour after such a procedure, abnormally low mitosis counts were frequently found. In experiments in which two hours or more were allowed to pass between these control procedures and the enucleation of the eyes for mitosis counts, no such abnormally low counts were found, and the general results of the experiments previously reported are in no way vitiated by this instability in short time control experiments. Nevertheless, since short time experiments were desirable for many purposes, an investigation of this matter was indicated. Experiments were therefore performed in order to see whether excitement would alter the mitotic activity of the corneal epithelium in a reproducible way and whether such an effect was in any way connected with the increased output of epinephrine caused by excitement.

METHODS. The method of counting mitoses in the rat's cornea has been described previously (4). The figures given below are the numbers of mitoses found in $\frac{1}{50}$ of the corneal area. In experiments designed to determine the mitotic rate as distinguished from the instantaneous number of mitoses present in the tissue, 3 mgm. colchicine per kgm. body weight was injected intramuscularly and the animals sacrificed 4 hours later.

I. Effect of excitement. Various stimuli were used to excite the rats: clapping

[1] This work was supported in part by a grant from The John and Mary R. Markle Foundation.

of hands near the cages, rattling or shaking the cages, hypodermic saline injections. The results with all of these were qualitatively the same. Pinching the animals' tails with a forceps yielded quantitatively the most reproducible results. As seen in table 1 there is a marked drop in the number of mitoses in the cornea 40 to 60 minutes after the animals had been excited, and the mitotic rate can be kept at a low level for as long as four hours by repeated stimuli.

II. *Effect of inhibitors of adrenergic actions on the responses to excitement.* In order to test whether this inhibition of corneal mitoses is related to a discharge of epinephrine, experiments were performed with two pharmacological agents which are known to interrupt the neuro-humoral mechanism of adrenergic actions at two different points: ergotamin and nicotin. Ergotamin inhibits some adrenergic actions at or near the site of the effector cell, while nicotin in suitable doses inhibits the output of epinephrine by paralysing the cholinergic synapse directly responsible for the stimulation of the adrenal medulla (7).

TABLE 1

Effect of excitement on corneal mitoses

Number of mitoses

	TIME AFTER ONSET OF 10–15 MINUTE EXCITEMENT PERIOD		
	0	40 minutes	60 minutes
Average mitosis counts	116	20	12
Number of eyes counted	50	8	8

Mitotic rate under colchicine

	CONTROLS	EXCITED FOR 10 MINUTES EVERY ½ HOUR
Average mitoses per hour	112	46
Number of eyes counted	19	21

One eye of each rat was removed under ether anesthesia. Two days later 15 mgm. ergotamin bitartrate per kgm. was injected intraperitoneally in one group of these animals, salt solution in another group. The animals were sacrificed one hour after the injection and the number of corneal mitoses compared with those in the previously removed eyes. The animals with salt solution showed a moderate decrease in mitosis count such as we had come to expect from this type of experiment. The animals with ergotamin showed on the average a slight increase in their mitotic counts. It appears then that ergotamin abolished and partially reversed the effect of excitement on the number of mitoses.

Similar results were obtained with nicotine. Each of a group of animals was given an intramuscular injection of 2 mgm. nicotin per kilo. Control animals received injections of salt solution. Twenty minutes after the injections their tails were pinched and they were kept excited for a period of 10 minutes' duration. They were sacrificed for mitosis counts 40 to 60 minutes after the onset of the excitement period. In the animals given saline injections plus excitement the

mitosis count averaged only 2 per cent of the normal, in those given nicotin plus excitement the count averaged 30 per cent of the normal.

In other experiments of longer duration we found that both ergotamin and nicotin have some depressing effect on the number of mitoses in the rat's cornea. The effect of these drugs is, therefore, complex, but this in no way contradicts the conclusion that the effect of excitement on corneal mitoses is through an adrenergic mechanism.

III. Effect of epinephrine. In order to avoid undesirable excitement effects rats were "conditioned" to the local instillation of eye drops by frequent application of saline eye drops for a period of about one month. At the end of this period epinephrine-acetate solution 1:1000 was instilled in one eye and a control-acetate solution in the other eye. A significant decrease in the number of mitoses occurred 40 to 60 minutes after a single instillation of epinephrine.

TABLE 2

Effect of systemic injection of epinephrine in peanut oil on corneal mitoses

Number of mitoses

	BEFORE INJECTION	HOURS AFTER INJECTION				
		1	2	4	6	8
Epinephrine						
Mitosis counts	100	4	1	33	19	18
Number of eyes counted	10	10	9	3	1	2
Peanut oil controls						
Mitosis counts	128	64	170			
Number of eyes counted	8	8	6			

Mitotic rate under colchicine

	EPINEPHRINE (MGM. PER KGM.)						PEANUT OIL CONTROLS	NORMAL CONTROLS
	1.0	0.5	0.25	0.1	0.05	0.025		
Average mitoses per hour	5	13	28	52	63	50	113	112
Number of eyes counted	2	2	2	2	2	2	22	19

The effect of systemic application of epinephrine is shown in table 2. In the first experiment shown in this table one eye each of some of the animals was removed as control, 2 to 3 days later injections were given of 1 mgm. epinephrine per kilo suspended in peanut oil. Controls were injected with peanut oil. The animals were sacrificed at various intervals after the injections for mitosis counts. The epinephrine produces an inhibition which is still marked eight hours after the injection. The peanut oil controls showed a slight drop in mitosis count at 1 hour, and recovery at 2 hours such as was to be expected from the excitement of the injection. The effect of epinephrine on the mitotic rate was tested by combining the injection of epinephrine with colchicine. The animals were sacrificed 4 hours after the injection. Doses as low as 0.025 mgm. per kilo produced a marked decrease in the rate of mitosis. The inhibition of mitosis by epinephrine is not due to a lowered temperature of the cornea from local vaso-

constriction since the inhibition can be demonstrated just as well in animals kept in a moist atmosphere in the incubator at 38°C. Moreover inhibition of mitosis can be produced by applying epinephrine to the enucleated eye kept in a moist warm chamber.

Supplementing our earlier observations on the effects of locally applied ephedrine (4), we investigated its effect on systemic application. In doses of 200 mgm. per kgm. bodyweight almost complete inhibition of mitosis occurs in the rat's corneal epithelium and the inhibition is still manifest 8 hours after injection.

IV. Effect of sympathectomy. In view of the results of our experiments with epinephrine it was of interest to see if the mitotic rate in the corneal epithelium is under the influence of local adrenergic innervation. Evidence of a more complex nature that sympathetic innervation exerts "trophic" influences has heretofore been presented for striated muscle (1, 2, 8), bone (6), skin (9) and cornea itself (3).

TABLE 3

Effect of removal of superior cervical sympathetic ganglion on corneal mitoses

Number of mitoses

	TIME AFTER DENERVATION				
	2½ hr.	5 hr.	19–20 hr.	1 wk.	2 wk.
Per cent difference denervated vs. control eye	+1	−15	+13	+18	−26
Number of animals	3	6	8	6	6

Mitotic rate under colchicine

	TIME AFTER DENERVATION					
	4 hr.	19 hr.	20 hr.	1 wk.	2 wk.	2½ wk.
Per cent difference denervated vs. control	+14	+11	−71	−52	−63	−57
Number of animals	5	4	3	5	8	10

PROCEDURE. Cervical sympathectomies were done on one side in rats under morphine or ether anesthesia. In one series of experiments the cervical sympathetic chain was torn preganglionically. In a second series of experiments the superior cervical ganglion itself was removed. In the rat this pear-shaped ganglion can easily be found medially and behind the bifurcation of the carotid artery if one gently pulls the artery laterally. Operative hemorrhage is rare, and postoperative infection is exceedingly rare in spite of an unsterile technique, but with the application of sulfanilamide powder into the wound.

Preganglionic sympathectomy is without any significant effect on the number or rate of corneal mitoses. After removal of the superior cervical ganglion there is also no marked or consistent change in the number of mitoses (table 3). When, however, colchicine is used to determine mitotic rates after ganglionectomy a marked and significant decrease is found. This decrease comes on sharply 20 to 24 hours after ganglionectomy and persists for a number of weeks thereafter.

The discrepancy between the experiments with and without colchicine, that is, between the rate at which cells enter mitosis and the instantaneous number found at a particular moment, is readily accounted for if we assume that removal of the superior cervical ganglion not only slows the rate of entrance into mitosis but also, and in approximately equal degree, slows the rate of progress through the mitotic cycle.

When the cervical sympathetic chain is interrupted either by preganglionic section or by removal of the superior cervical ganglion ocular symptoms,—miosis, ptosis, enophthalmos—become manifest almost immediately. On the other hand the decline in mitotic activity following removal of the ganglion appears only after a lag of some 20 hours. This interval corresponds approximately to the time required for nerve degeneration to produce a local hypersensitivity to epinephrine. The question arises, therefore, whether the decrease in mitotic activity may be merely an evidence of local hypersensitivity to circulating epinephrine. Against this are the following arguments:

1. The decreased mitotic activity was found to be just as great when extreme precautions were taken to avoid exciting the rats during the 4 hour interval between injection of colchicine and the end of the experiment. (The animals with these special precautions were the group "2 weeks" in table 3.) 2. If there were enough circulating epinephrine to produce an inhibition due to local hypersensitivity other local hypersensitivity reactions to epinephrine, e.g., dilatation of the pupil and widening of the lid slit would also be expected to have occurred, but these were not found. 3. In none of the many epinephrine experiments reported above have we found evidence of a slowing down of the mitotic cycle,—only an inhibition of the onset of mitosis. Consequently it seems unlikely that the effect of removal of the superior cervical ganglion is to be explained as a hypersensitivity reaction to circulating epinephrine. Rather it would seem that the operation has deprived the corneal tissues of some factor necessary for the maintenance of normal mitotic activity. This normal requirement cannot be sympathin since sympathin is lacking in the ocular tissues after preganglionic sympathectomy which is without effect on the mitotic rate.

This argument need not be taken as a denial of any relationship between mitotis decrease and hypersensitivity to epinephrine in the same tissue. Cannon and Rosenblueth (5) have pointed out that the hypersensitivity to epinephrine is not highly specific and that hypersensitivity to some other drugs occurs simultaneously in sympathectomized tissues. Kesselring (9) has found evidence of decreased cellular permeability after sympathectomy. Decreased mitotic activity and hypersensitivity to epinephrine may therefore both be similarly dependent upon the complex results of denervation.

General discussion. It is of interest whether the mitotis inhibition by locally or systemically applied epinephrine and by excitement is a general

locally or systemically applied epinephrine and by excitement is a general

alimed sections of the bone marrow and small intestine of some animals following the administration of epinephrine. In the absence of satisfactory methods of mitosis assay on these organs our findings can have little significance. On the average there were fewer mitoses in these tissues than in normal controls, but

certainly no such profound inhibition of mitosis as was found in the cornea. It is evident that various tissues react differently to the mitotis inhibiting effect of epinephrine.

von Haam and Cappel (10) reported that epinephrine in concentrations of from 10^{-9} to 10^{-4} inhibits the growth of tissue cultures of heart fibroblasts, as gauged by surface area measurements, and also decreases the mitotic index. The concentrations used in our systemic experiments fell within this range; computed for the bodyweight of the rats, they were between 2.5×10^{-8} and 10^{-6}, and considering the slow absorption of epinephrine in oil, it would appear that the active concentrations in the tissue were still somewhat lower. The experiments of von Haam and Cappel show also that other tissues as well as the corneal epithelium are—at least under certain conditions—susceptible to the mitosis inhibition by epinephrine.

The experiments on sympathectomized rats were designed originally as one test for the rôle of "neurotrophic" influences since the susceptibility of mitosis to epinephrine suggested the possibility of a sympathin-mediated growth-regulating effect. Much to our surprise, sympathectomy did not produce an increased, but a decreased mitotic rate.

SUMMARY

1. Excitement or annoyance of rats, particularly by painful stimuli, diminishes the mitotic rate in their corneal epithelium.

2. The decrease of mitotic rate caused by excitement is an adrenergic response and can be simulated by local or systemic application of epinephrine. Both ergotamin and nicotin diminish the mitosis inhibition following excitement. The effect of epinephrine is not due to a decrease in tissue temperature or to local circulatory disturbances.

3. Removal of the superior cervical ganglion leads to a decreased mitotic rate in the rats' corneal epithelium after a lag period of about 20 hours. It is suggested that this effect is attributable to the absence of some factor other than sympathin which regulates mitotic activity in this tissue under physiological conditions, and that this manifestation of denervation may be related to that of sensitization.

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DETERMINATION OF BLOOD AND PLASMA VOLUME PARTITIONS IN THE GROWING RAT[1]

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Received for publication April 10, 1944

Proper evaluation of experimentally induced alterations in the components of the extracellular compartment is largely dependent upon recognition of changes in blood volume partitions. It might be expected that such changes would occur during the process of normal growth. Despite the common use of the laboratory rat in physiological, biochemical and nutritional experiments, alterations in its blood and plasma volumes have been, for the most part, ignored. Since the factors contributing to this omission are the scarcity of reported methods, their technical difficulties or inherent errors, the method described in this communication is believed to be of value. Simple, multiple and relatively accurate determinations are possible.

Reported methods for determining plasma volume are of three types: *1*, complete washing-out technique, originated by Welcker (1858) (1), used by Jolly and Stini (1905) (2), by Chisholm (1911) (3), and recently modified by Cutting and Cutter (4); *2*, gasometric (CO) dilution method of Scott and Barcroft (5); and *3*, the dye dilution method originated by Keith, Rowntree and Geraghty (6), first applied to rats by Cartland and Koch (7), with subsequent modifications by Went and Drinker (8) and Griffith and Campbell (9). In each of these instances the dye Brilliant Vital red was used. The only available report in which the diazo blue dye T-1824 has been used is that of Beckwith and Chanutin (10).

Values obtained by the methods noted above are summarized in table 1. We have obtained somewhat higher values for total blood volume and plasma volume in a series of thirty-four male rats, approaching that range (8 to 10 per cent body weight) found in other animals and in man (11, 12, 13, 14 and 15). These values have been adjusted, at three different stages of growth, to both surface area and body weight. Apparently, large mature rats have relatively smaller unit blood volumes per unit mass than do small, maturing rats (7, 9). This phenomenon has not been noted in dogs or man (11). There is, however, an absolute increase of total blood and total and unit plasma volume in older, larger animals as was observed by Cutting and Cutter (4).

METHOD. Young, weanling (40 grams), Sherman strain rats were placed in individual cages and fed a synthetic, purified and presumably adequate diet composed of

Sucrose	73 per cent
Casein (vitamin free)	18 per cent
Phillips and Hart salt mixture IV	4 per cent
Corn oil (Mazola)	5 per cent

[1] Aided in part by a grant from the William W. Wellington Memorial Fund.

with the water soluble vitamins, B_1 (200 γ), B_2 (400 γ), B_6 (200 γ), niacin (2500 γ), calcium pantothenate (1500 γ) and choline (100 mgm.) added per 100 grams ration. Supplementary feedings of the fat soluble vitamin concentrates haliver oil, viosterol and α-tocopherol were administered in appropriate amounts by syringe *per os* biweekly. Water was taken *ad lib.* The daily growth increment of 3 to 4 grams per day may be considered normal for the strain and diet. Surface area was determined by Lee's formula (16):

$$\text{Surface Area (cm.}^2) = 12.54 \text{ Weight}^{0.60}$$

After one week on the experimental diet, arbitrary division into groups was made.

Group I—Composed of twelve animals, weighing 41.4 to 68.3 grams with

TABLE 1

AUTHOR	METHOD	NUMBER OF RATS USED	TOTAL PLASMA VOLUME	*PV*/100 cm²	BLOOD VOLUME %
Jolly and Stini (1905)	Perfusion				4–5 cc.
Chisholm (1911)	Perfusion	25			5.3–7.3 cc.
Cutting and Cutter (1935)	Perfusion	25	5.04–5.46—small rats 7.53–10.8 —large rats	1.4 cc.	
Barcroft (1924)	CO	24			5.4–7.9 cc.
Cartland and Koch (1928)	2% Brilliant vital red 0.25 cc.—samples at 1.5–2.0 min.	7			6.2–7.7—small 6.2–6.9—large
Went and Drinker (1929)	Brilliant vital red, samples at 4.5 min.	9			7.4% body weight
Griffith and Campbell (1937)	5% Brilliant vital red, samples at 4.5 min.	20			4.1–5.3
Beckwith and Chanutin (1941)	1% T-1824 extrapolation	53	Complete data not available	2.71 ±.09	7.98 ±0.85 5.37 ±0.41/100 cm.
Metcoff and Favour (1944)	0.1% T-1824 samples at 3.75–4.25 min.	34	2.19–9.99 varies with surface area	2.90 ±.09 varies with surface area (age)	Varies with mass and amount of fat 7.2–10.6% body weight

surface area of 116.7 to 158.1 sq. cm. Plasma volumes[2] were determined after 2 weeks on the experimental diet.

Group II—Composed of nine animals whose plasma volumes were determined after 24 days on the experimental diet. They weighed 73.3 to 89.1 grams with surface area equal to 165.1 to 185.5 sq. cm.

Group III—Composed of nine animals from groups I and II (30 days having elapsed since the last plasma volume determination) and four animals from a previous group maintained on the same diet, under identical conditions, with a similar growth increment. These rats weighed 137.2 to 335.8 grams with surface area equal to 240.4 to 413.1 sq. cm.

[2] In addition to plasma volume, hemoglobin, hematocrit and total protein determinations were made in each instance to provide coincidental control data for an associated study to be reported at a later date.

PROCEDURE. After being fasted for 4 hours, the rat is carefully weighed and then etherized, with minimal excitation, in a large, cotton-padded jar. Anesthesia is maintained by means of drop ether and a small paper cone partially packed with ether-moistened cotton. The cone, applied over the snout, is held *in situ* by wire or a straight pin which penetrates it from side to side and is snagged behind the upper incisors. A fairly large-mouthed cone affords proper aeration and a drop or two of ether on it from time to time suffices to maintain an even plane of anesthesia.

The animal is fastened to a board in such fashion that the anterior aspect of the neck is hyperextended. The area over the right or left external jugular is carefully shaved from clavicle to mandible and a 1.5 cm. incision through the skin is made. The external jugular is readily exposed and divested of its sheath from its point of emergence in the supraclavicular space (jugular bulb) to its cephalad bifurcation. The dye is injected with a calibrated syringe adjusted to deliver between marks. This avoids "dead-space" errors. Two-tenths cubic centimeter of 0.1 per cent aqueous solution of T-1824[3] is used in animals weighing less than 150 grams; 0.4 cc. in larger animals. The dye is injected through a no. 26 needle inserted into the vein so that its point lies free within the jugular bulb. The dye may be observed as it enters and mixes with the blood stream. A time clock is started immediately upon completion of rapid injection, but the needle is not removed from the vein until 30 seconds have elapsed. There is no leakage. Three to 3.5 minutes are allowed for adequate mixing after which time 0.6 to 0.8 cc. blood is obtained by heart puncture between 3.75 and 4.25 minutes following injection. This blood is immediately transferred to shortened precipitin tubes containing 0.2 mgm. of liquid heparin which had been previously evaporated to dryness at 37°C. Two-hundredths cubic centimeter of blood is removed with a Sahli pipette and delivered into a graduated Klett tube containing 5 cc. distilled water. Hemoglobin and hematocrit estimations are to be made from this sample. The remainder of the blood is then centrifuged at 2500 r.p.m. for 30 minutes. The dyed plasma is removed and kept in a stoppered tube at 8°C.—a small portion of the plasma remains trapped between the red blood cells (17, 18) and would contribute to the inaccuracy of the conventional hematocrit. Concentration of dye in the sample is always measured before 24 hours have passed. The mortality of the operative procedure is 2.5 per cent. Death, when observed, results from traumatic heart puncture and subsequent cardiac tamponade.

Determination of plasma volume. Any determination of plasma volume by the dye dilution method is dependent upon several factors. These are 1, the time required for complete mixing; 2, the rate of disappearance of the dye from the blood stream, and 3, the accuracy of the method used for measuring concentration of the plasma diluted dye. In a method which attempts to estimate plasma volume from a single sample, that sample must contain a dilution of dye approximating its initial concentration in the plasma. This, in turn, is dependent upon the time required for mixing and the amount of dye lost during this period (19).

[3] We wish to acknowledge the kindness of Dr. John G. Gibson, 2nd, who supplied us with the dye.

The concentration changes during the several minutes after injection afford the maximal source of error. Backward extrapolation of several samples obtained at different intervals avoids this and, although subject to numerous errors, is feasible in the rat (10). Investigators differ, however, in deciding which particular straight line should be drawn through the time-concentration curve to indicate the initial concentration. It has been suggested that this difficulty is obviated if concentration is plotted against the square root of time (20). Gregersen, on the other hand, points out that the time-concentration curve is more precisely described as a logarithmic function, since the amount of dye escaping per unit time must be a constant fraction of the amount of dye remaining (19). Reference to the dot diagram (fig. 1) in which the logarithm of the relative optical density (concentration) is plotted against time, indicates that large discrepancies in apparent dye concentration may occur if blood is drawn before 1.5 minutes have elapsed. No data are available for the rate of disappearance of T-1824 from the rat's circulation, but vital red is reported to leave this animal's blood stream at approximately 11 to 12 per cent per hour (9). Since both dyes have a similar slow rate of disappearance (19), one may assume that T-1824 concentration decreases at approximately 9 to 12 per cent per hour. This is slightly higher than the rate as reported in dogs. An arbitrary sampling time of 3.75 to 4.25 minutes probably reflects the initial dye concentration, and ± 1 minute would result in an error of ± 0.1 per cent in total plasma volume where $200\,\gamma$ of dye are injected.

The dye is non-toxic and does not pass the glomerular membrane, but is removed by the liver (via the bile) and by reticulo-endothelial cell activity and storage (21, 22). In dogs it appears in the lymph within 1 hour (23). T-1824 is firmly bound to the albumin fraction and usually migrates with it in the electrophoretic field (24). Although 0.4 to 1.4 mgm. of protein per 12 hours per 100 sq. cm. body surface is excreted in the urine of the rat, this protein may be of genital, not renal, origin (25).

Estimation of total blood volume. Estimation of the total blood volume is dependent upon an accurate estimation of the cell volume. A conventional hematocrit determination requires too much blood, and the several procedures evolved to obviate this difficulty are either complicated or prone to error. Ashworth and Tigertt (26) and Brown (27) have shown that accurate estimations of cell volume and hemoglobin concentration could be made from the specific gravities of whole blood and plasma, since the weight of 100 cc. of blood is a function of the sum of the weights of its cells and plasma. Formulae for calculation are based upon the assumption that cell gravity and hemoglobin concentrations within the cells are nearly constant or that changes in cell gravity and cell hemoglobin are usually parallel. Utilizing this constant, determined by conventional centrifugation,

$$\text{Hematocrit} = 100 \times \frac{\text{Gravity blood} - \text{Gravity plasma}}{1.0970 - \text{Gravity plasma}}$$

a gravometric procedure such as this would require at least 0.3 cc. of blood. Van Slyke et al. (28) point out in their monograph that if the hemoglobin con-

centration of the cells is fairly constant, as is their gravity, one may substitute the O_2 capacity found per 100 cc. of cells in the above equation and thereby calculate hemoglobin in terms of O_2 capacity. Bernhart and Skeggs (29) have shown that 1 gram of hemoglobin binds 1.36 cc. O_2; therefore,

$$\frac{O_2 \text{ capacity}}{1.36} = \text{gram hemoglobin.}$$

Van Slyke et al. determined that the O_2 capacity of hemoglobin per 100 cc. of cells is 46.1 in man—hence

$$\text{Hemoglobin (grams/100 cc.)} = 33.9 \times \frac{\text{Gravity blood} - \text{Gravity plasma}}{1.0970 - \text{Gravity plasma}}$$

The similarity of these equations for hemoglobin and hematocrit estimation is evident, and if the assumptions made are valid in the rat, knowing the value for hemoglobin in gram per 100 cc., the cell volume may be estimated by substitution, where:

$$\frac{\text{Gravity blood} - \text{Gravity plasma}}{1.0970 - \text{Gravity plasma}} = \frac{\text{Hemoglobin (grams per cent)}}{33.9}$$

and

$$\text{Hematocrit} = 100 \times \frac{\text{Gravity blood} - \text{Gravity plasma}}{1.0970 - \text{Gravity plasma}}$$

$$\therefore \quad \text{Hematocrit} = \frac{\text{Hemoglobin} \times 100}{33.9} \quad \text{or}$$

$$\text{Hematocrit} = 2.95 \times \text{Hemoglobin}$$

Hemoglobin determinations are easily made using the photoelectric colorimeter with a filter having maximal absorption at 540 $\mu\mu$.

These determinations require only 0.02 cc. of whole blood (diluted in 5 cc. distilled water). The presence of dye in the plasma does not interfere appreciably with the hemoglobin determinations.

Cell volumes estimated in this fashion compare favorably in the rat, as they do in man and dogs, to those obtained by conventional centrifugation. The error is ± 0.4 per cent. Calculation, using this method, of mean hematocrit values from the data on seventy-three rats reported by Wintrobe, Shumacker and Schmidt (30), agrees quite well with their observed values.[4] Although calculations are based upon Newcomber hemoglobin determinations as reported, the error between observed and calculated results is only 2.5 per cent.

Standardization. Dye dilution methods require that standards of known dye concentration be prepared for comparison with the unknown. When the photoelectric colorimeter is used, it is necessary to know only the relative optical densities of known concentrations of dye from which the factor of the filter may be calculated. The relative optical density and dilution of the dye in the unknown plasma sample may then be determined. Standardization (determina-

[4] Hemoglobin (Newcomber) = $13 \pm 0.08\ \sigma = 1.17$.
Hemotocrit (conventional, observed) = $39.4 \pm 0.23\ \sigma = 3.62$.
Hematocrit (calculated) = 38.4. Error = 2.5 per cent.

tion of the factor F) should be made at a range approximating that of the unknown and two standards

$$S_I = 50\gamma \text{ dye/cc.}$$

$$S_{II} = 100\gamma \text{ dye/cc.}$$

have been quite satisfactory. S_I is prepared by delivering 0.1 cc. of 0.1 per cent dye (100γ) from a carefully calibrated delivery pipette into 1.80 cc. of 0.85 per cent saline contained in a Klett microtube. To this is added 0.1 cc. of undyed control rat plasma for a total of 2.0 cc. S_{II} is prepared in similar fashion, except that 0.2 cc. (200γ) dye and 1.70 cc. of saline with 0.1 cc. of plasma are used. A plasma blank containing 0.1 cc. plasma plus 1.90 cc. saline is prepared for every series of determinations. The relative optical densities of these known dye concentrations are measured with a color filter transmitting light in the region of $620\mu\mu$. This is the band of maximal absorption for the dye T-1824. Transmission spectra of oxyhemoglobin are maximally absorbed at a wave length of $540\mu\mu$ and therefore slight hemolysis does not significantly interfere (31). The unknown is prepared by delivering 0.10 cc. of the dyed plasma sample into 1.90 cc. of 0.85 per cent saline in the microtube. All pipettings must be made with great accuracy from calibrated standardized delivery pipettes.

Calculations. For the Klett-Summerson photoelectric colorimeter:

Concentration dye = Reading (= Relative optical density) × Factor. Knowing both the concentrations and relative optical densities of S_I and S_{II} the value of factor F may be determined. The value so obtained will vary slightly from instrument to instrument. The average value of F derived from the relative optical densities of 10 standards S_I and S_{II} was found to be 1.097. Using this factor, total plasma volume in cubic centimeters may be calculated directly according to the formula,[5]

$$PV_{\text{Total (cc.)}} = \frac{\text{Concentration of dye injected}}{\text{(Relative optical density)(Factor)}}$$

$$PV_{\text{Total (cc.)}} = \frac{200}{R(1.097)}$$

or, where 400 γ dye injected

$$= \frac{400}{R(1.097)}$$

Total blood volume is calculated according to the formula:

$$BV_T = \frac{PV_T}{100 - Ht.}.$$

Total cell volume,

$$CV = BV_T - PV_T.$$

[5] We wish to express our appreciation to Dr. Otto Schales for valuable assistance in the derivation of these formulae as they apply to the Klett.

Values obtained for total plasma volume (PV_T) and total blood volume (BV_T) are adjusted to unit plasma volume (PV/100 cm.²S.A.), and BV/per cent body weight. In the latter instance,

$$BV \text{ per cent b.w.} = BV/100 \text{ grams} \times 1.06,$$

where 1.06 represents the specific gravity of adult rat whole blood. In young rats (less than 100 grams) this factor is 1.05.

TABLE 2

Relation of relative plasma optical density/200 γ dye, surface area, and time of sampling

TIME	SURFACE AREA (CM²)											
	120–159			160–219			220–299			300+		
	R	*R(F)*	Log.	*R*	*R(F)*	Log.	*R*	*R(F)*	Log.	*R*	*R(F)*	Log.
minutes												
1–1.5	34.8	37.1	1.569				17.5	19.2	1.283			
	21.8	23.9	1.378									
	21.8	23.9	1.378									
2							25	27.4	1.438			
2.75							16.5	17.1	1.233			
							40.0	43.8	1.642			
							26.0	28.5	1.455			
3.5–4.5	62	68.0	1.833	41	44.9	1.652	27	29.6	1.471	25	27.4	1.438
	51	55.9	1.747	35	38.3	1.583	20	21.9	1.340	18	19.7	1.255
	81	88.8	1.948	30	32.9	1.517	35	38.3	1.583	20	21.9	1.340
	42	46.0	1.663	39	42.7	1.630	25	27.4	1.438	21	23.0	1.322
	60	65.8	1.818	36	39.4	1.596	19	20.8	1.279			
	58	63.5	1.803	30	32.9	1.517	20	21.9	1.340			
	65	71.2	1.853	39	42.7	1.630	27	29.6	1.471			
	74	81.0	1.909	36	39.4	1.596						
	73	80.0	1.903	29	31.8	1.462						
5.5	60	65.8	1.818									
6+	68	74.5	1.833									

Where:
R = Relative optical density/200 γ dye injected,
F = 1.097.

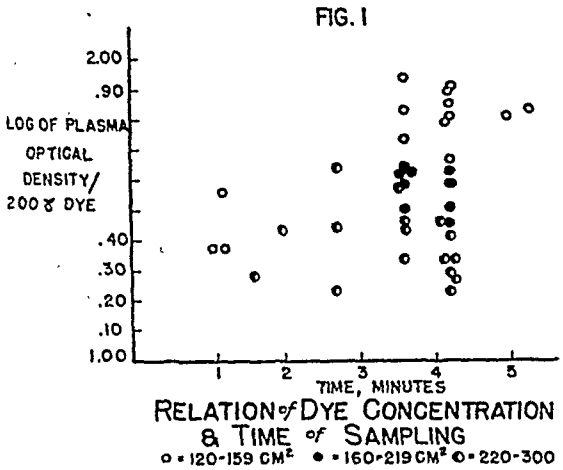
OBSERVATIONS. Relation of relative optical densities to time of sampling and surface area is noted in table 2, and depicted graphically in the dot diagram, figure 1. It will be noted that comparatively good grouping of relative optical densities according to size is obtained between 3 to 5 minutes. There is greater variation if samples are obtained earlier, being most marked if obtained within 1.5 minutes after dye injection. This represents inadequate mixing. Three to 5 minutes, therefore, probably represent the time of complete mixing. It is un-

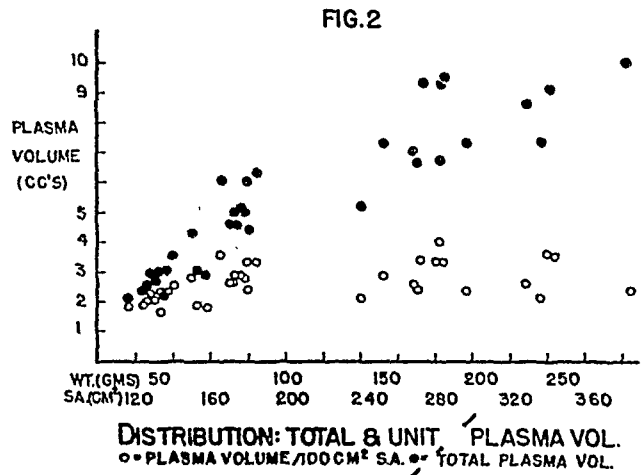
TABLE 3

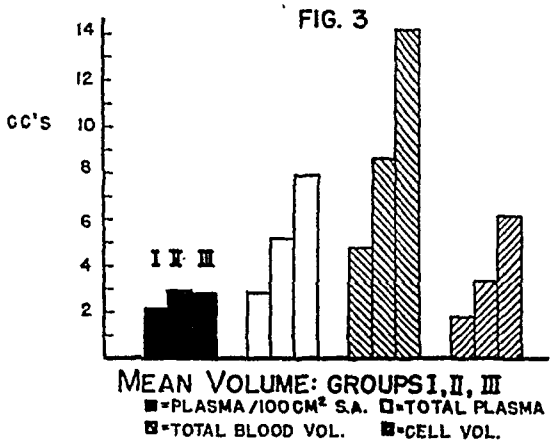
GROUP	WT.	S.A.	PV_T	PV/100 cm.²	Ht.	BV_T	BV%BW
	grams	*cm.²*	*cc.*			*cc.*	
I	41.4	116.7	2.19	1.88	33.1	3.35	8.8
	45.4	123.8	2.45	1.99	37.5	3.91	9.1
	45.6	124.2	2.50	2.00	35.7	3.88	8.9
	48.7	129.1	2.94	2.26	33.9	4.42	9.5
	49.8	130.1	2.69	2.05	39.9	4.46	9.5
	51.7	133.2	3.03	2.27	40.4	5.07	10.4
	52.3	134.5	2.25	1.68	41.3	3.82	7.5
	53.4	136.2	3.14	2.30	33.6	4.72	9.4
	56.3	140.9	3.56	2.53	38.6	5.81	10.9
	63.1	150.8	4.32	2.86	36.6	6.82	11.6
	64.6	152.9	3.03	1.98	34.2	4.61	7.5
	68.3	158.1	2.81	1.76	40.7	4.72	7.4
Mean	53.4	136.5	2.98	2.13	37.1	4.75	8.9
σ distribution			±0.57	±0.32		±0.91	±1.08
II	73.3	165.1	6.07	3.66	33.6	9.10	13.1
	77.6	170.8	4.57	2.65	36.9	8.10	11.0
	78.0	171.3	4.57	2.65	36.0	7.15	9.2
	79.0	172.6	5.06	2.93	40.7	8.50	11.4
	81.4	175.6	5.21	2.96	36.0	8.15	10.0
	83.4	178.2	5.06	2.73	37.4	8.04	10.4
	83.7	178.6	6.07	3.39	32.7	9.00	11.3
	84.9	180.1	4.45	2.46	31.8	6.50	8.4
	89.1	185.5	6.29	3.37	37.7	10.01	11.9
Mean	81.2	175.4	5.27	2.99	35.8	8.67	10.6
σ distribution.....			±0.67	±0.38		±1.07	±1.32
III	137.2	240.4	5.22	2.16	41.0	8.78	6.4
	148.7	252.2	7.34	2.89	39.3	12.00	8.6
	165.9	269.4	7.01	2.60	47.5	12.28	8.4
	167.0	270.5	6.72	2.49	46.9	12.70	8.3
	168.2	271.6	9.31	3.48	39.5	15.50	9.7
	177.2	281.1	9.31	3.31	45.7	17.10	10.1
	178.6	281.4	6.75	2.40	48.4	13.10	7.5
	179.8	282.6	9.58	3.37	42.8	16.70	10.1
	194.4	296.1	7.30	2.46	41.6	12.50	6.8
	231.4	328.8	8.65	2.64	45.4	15.90	7.3
	241.3	337.2	7.34	2.16	44.3	13.10	5.8
	246.2	341.3	9.10	2.67	46.0	16.90	7.3
	335.8	413.1	9.09	2.45	41.7	17.40	5.5
Mean	197.9	299.2	7.97	2.77	43.9	14.21	7.2
σ distribution.....			±1.87	±0.46		±2.48	±1.60

* BV per cent $B.W.$ = BV/100 g × 1.06. BV per cent $B.W.$ of 34 animals = 8.9% ±0.245; σd = ±1.43. PV/100 cm² of 34 animals = 2.90 ±0.085; σd = ±0.50.

likely that an appreciable amount of the dye would escape from the plasma during this interval. The data on blood and plasma volumes are contained in table 3, and graphically illustrated in figures 3 and 4. The differences between the mean values for the various blood partitions in the different weight groups is quite







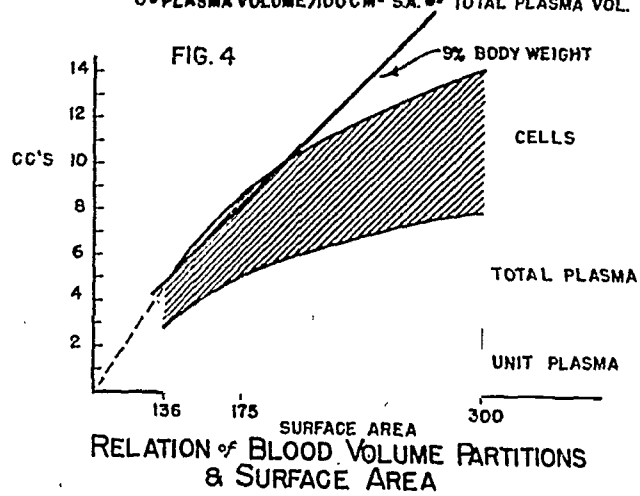


Fig. 1. Comparison of the relative optical density caused by dilution of the dye in the plasma of the three different sized groups of animals in relation to the time at which the plasma sample was obtained.

Fig. 2. Distribution of the observed total and unit plasma volume observations in thirty-four animals according to weight and surface area.

Fig. 3. Comparison of the mean values obtained for the various blood volume partitions in the three different sized animals.

Fig. 4. A diagrammatic representation of the changes observed in the various blood volume partitions as the animal increases in size. The total blood volume is the sum of the total plasma and cell volumes. A line representing 9 per cent of body weight has been drawn in order that the total blood volume may be compared with it.

apparent. Figure 2 is a dot diagram showing distribution of total and unit plasma volumes relative to body weight and surface area. Probable significance of the difference between the unit plasma volume and per cent body weight means of groups I, II and III were determined according to the method of small number analysis (32).

DISCUSSION. Inspection of the data in table 3 indicates, as would be expected, that total plasma, cell and blood volumes are related to body size. This relationship is not linear. It is interesting, however, that unit plasma volume (cc./100 cm.^{2}) attains its maximal value in adolescent 70 to 90 gram rats (group II). Application of the "t-test" (32) indicates that the observed increases are probably significant. The apparent decrease in unit plasma volume of group III is not statistically significant.[6]

Consideration of total blood volumes as constituting a given percentage of body weight has been subjected to much valid criticism by most workers. Despite these objections, and since much of the existing literature on blood volumes has been reported on such a basis, these values were calculated. A significant difference was found to exist between the blood volumes, expressed as per cent of body weight, in each of the three groups of rats.[7] Group II would appear to contain more blood per cent of mass than either of the other groups. This observation is of some interest in view of the fact that 70 to 90 gram growing rats are pubescent. The data for children suggest the transient occurrence of a similar event (13). It is quite possible, however, that the expression of blood volume per cent body weight in the largest animals errs because of the larger proportion of body fat to total mass, as pointed out in dogs (11) and humans (33). Such an error would result in an apparently low value. A minimal increase of cell volume relative to total blood volume is noted in group II (37.3:38.8 per cent = 1.5 per cent more than group I). In the interval between the periods of growth represented by groups II and III, the cell volume attains its adult relationship to the total volume—increasing 5 to 6 per cent over the previously observed values. It would appear to attain its maximal unit volume later than does the plasma.

It is evident from the progressive changes in the data of groups I, II and III that it is neither logical nor valid to offer a single expression for total or unit blood, cell or plasma volumes. The relationship of that expressed value to body size and the approximate stage of growth—young, pubescent or mature—should be specified.

Comparison of the results obtained with the previous available data on volume studies in the rat is difficult owing to the inadequacy of published data. In general it may be stated that the values herein reported for total blood and plasma volumes agree with those reported by Cutting and Cutter, and Beckwith and Chanutin. The mean unit plasma volume of the entire group, although it is probably inaccurate to express such a value, agrees favorably with that reported by Beckwith and Chanutin, also using T-1824. The low value of Cutting and

[6] σ^2 difference of mean unit PV_{II} and $PV_{III} = \pm 0.495$, $t = 0.33$, $n = 21$, and P = less than 0.70; therefore not significant.

σ^2 difference of mean unit PV_I and $PV_{III} = \pm 0.425$, $t = 2.93$, $n = 23$, and P = less than 0.01; therefore probably highly significant.

[7] σ^2 difference of BV per cent I and II $= \pm 1.92$, $t = 280$, $n = 19$, and P = less than 0.02; therefore significant. σ^2 difference of BV per cent II and III $= \pm 2.46$, $t = 5.50$, $n = 20$, and P = less than 0.01; therefore significant.

σ^2 difference of BV per cent I and III $= \pm 2.55$, $t = 2.98$; $n = 23$, and P = less than 0.01; therefore significant.

Cutter may be dependent upon their method of computing surface area. Expressed as per cent of body weight, the present data agree quite well with those of Beckwith and Chanutin, Went and Drinker, and those reported in other species (dog, man). They are somewhat higher than those reported by other workers measuring rat blood volumes.

The washout and gasometric techniques preclude a chronic experiment. Use of a red dye involves a definite subjective error in ordinary colorimetric analysis, plus an attendant hidden error resulting from hemolysis. The extrapolation method using T-1824 would appear to yield excellent results, but is somewhat cumbersome and requires a rather large amount of total blood. The apparently small amount, 0.2 cc., required at each bleeding may after several withdrawals produce a significant change in the extravascular and plasma partitions in an endeavor to maintain homeostasis. Extrapolation methods, especially in small animals, may measure an indeterminate portion of lymph volume in addition to plasma volume (34).

SUMMARY. The scarcity of adequate available complete data on blood and plasma volume in the rat prompted this investigation. Plasma dilution of the diazo blue dye T-1824 seemed to offer the best method, at the present time, for determining these values with any degree of accuracy. This method has an obvious advantage in chronic experiments where repeated determinations are desirable. A single small sample of plasma diluted dye was obtained by heart puncture. From it, using the Klett-Summerson photoelectric colorimeter, plasma, blood, and cell volumes were determined in thirty-four male rats. A new method, which may possibly be of some value, was suggested to determine cell volume per 100 cc. (hematocrit). It is necessary in rats, as in other animals, to consider the blood volume partitions in relation to body size and the stage of growth.

CONCLUSIONS

No definite conclusions may be drawn from data derived from a relatively small number of observations; however, the following suggestions may be made:

1. Use of the diazo blue dye T-1824, the photoelectric colorimeter, and a single sample technique afford a relatively accurate and simple method for determining plasma volume in the rat.
2. Cell volume per 100 cc. blood (hematocrit) may be estimated from the hemoglobin concentration.
3. A significant increase in total blood, plasma and cell volumes is associated with growth and change in body size. This increase is not linear.
4. A significant increase in unit plasma volume (cc./100 cm.^{2}) also appears to be associated with apparently normal growth.
5. Unit plasma volume attains its maximal value in 70 to 90 grams pubescent rats; unit cell volume, in larger more mature animals.
6. Seventy to 90 grams growing rats would appear to have larger unit blood volumes (expressed as per cent body weight) than either smaller or larger rats. This actually may not be the case, since larger animals have more body fat.

7. The trend and relative range of these observations are similar to those observed in dogs and children.

Acknowledgments. The authors wish to acknowledge the valuable technical assistance of Mrs. Jean Pfeffer, laboratory technician, for her work done in connection with this paper.

We are indebted to Drs. G. W. Thorn, C. K. Drinker, J. G. Gibson, 2nd and F. J. Stare for their valuable suggestions and criticisms concerning its preparation.

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THE EFFECTS OF NEMBUTAL AND YOHIMBINE ON CHRONIC RENAL HYPERTENSION IN THE RAT

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Received for publication April 11, 1944

Since Goldblatt and his co-workers showed that partial occlusion of the renal arteries in dogs (1), monkeys (2) and other species (3) may result in permanent arterial hypertension, the mechanism of this hypertension has been elucidated by many investigators. Their work has shown that the appearance of this phenomenon is not dependent on the nervous system or the endocrine system (with the possible exception of the adrenal cortex), but is due to a disturbance of a humoral mechanism in the kidney. Admirable reviews of the evidence leading to this conclusion have been presented by Lewis and Goldblatt (4), Page (5) and Braun-Menendez (6).

On the other hand certain observations are difficult to reconcile with the accepted view that the hypertension induced by renal "ischemia" is *maintained* by the renal pressor system.

The observations of Taggart and Drury (7) on the similarities of the responses of normal and hypertensive rabbits to renin, and their conclusion that some other factor than renin is involved in the maintenance of hypertension, have never been satisfactorily explained. Dock's finding that normal and hypertensive rabbits show similar low blood pressures after pithing (8) implicates a nervous rather than a humoral mechanism. The effects of unilateral nephrectomy on single ischemic kidneys in hypertensive rats have been investigated by three groups of workers (9), (10), (11). All of these report that in the majority of cases the blood pressure is not lowered by nephrectomy. Patton, Page and Ogden (9) have succeeded in establishing an inverse relationship between the duration of the hypertension and the lowering of the blood pressure by unilateral nephrectomy. Even bilateral nephrectomy fails to lower the blood pressure of chronically hypertensive rats (12), (13).

The critical experiment in support of the humoral theory of hypertension—the demonstration of an alteration in the elements of the humoral system in the hypertensive animal—has yielded contradictory results in the hands of several investigators. Using similar techniques some workers (14), (15), (16) have found pressor substances in the blood of hypertensive animals, while others (17), (18), (19) have not. Unfortunately in these experiments the factor of the duration of hypertension is rarely discussed.

Some of these difficulties and inconsistencies may be resolved by modifying the purely humoral theory of hypertension with the concept that *the mechanism of hypertension changes qualitatively with time.* Thus a hypertension originally

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precipitated by a disturbance in the renal pressor mechanism might persist after the disturbance had been removed as the result of alterations in the neurovascular set (Dock).

The nervous system has apparently been exonerated in renal hypertension (see the reviews cited). On the other hand the interpretation of the fact that hypertension can be maintained in animals after nerve section and removal must be carefully re-evaluated in view of the fact that nervous control over peripheral vessels has been shown to return with extraordinary rapidity (20), after sympathectomy, and that even undoubted neurogenic hypertension produced by moderator nerve section is only temporarily abolished by complete sympathectomy (21).

In the present experiments we have reinvestigated the rôle of the nervous system in the maintenance of hypertension, using the rat as the experimental animal. The brief life span of this species afforded the possibility that whatever changes occurred in the mechanism of hypertension would be accelerated, and the evidence already cited that unilateral and bilateral nephrectomy fail to lower the blood pressure of rats hypertensive for more than two months to normal levels afforded a convenient reference point in time for the study of changes in the mechanism of hypertension.

METHODS. Rats were made hypertensive by partial unilateral occlusion of the renal artery according to the method of Wilson and Byrom (22). Systolic blood pressure was measured with the tail plethysmograph of Williams, Harrison and Grollman (23) and no rat was considered hypertensive unless it had maintained an average blood pressure of 150 mm. Hg or more before use. All normal controls were from the same stock and of about the same age as the hypertensives.

After hypertension had been maintained for one day to four months, the effects of the following substances on the blood pressure were determined: nembutal, 40 mgm. per kilo; nembutal, 20 mgm. per kilo; nembutal, 40 mgm./kilo and picrotoxin, 4.5 mgm. per kilo administered together; yohimbine hydrochloride, 3.0 mgm. per kilo. All substances were administered intraperitoneally.

FINDINGS. The findings are summarized in table 1.

Nembutal anesthesia. Nembutal (40 mgm. per kilo) was injected intraperitoneally. The pressure was taken just before and 15 to 30 minutes after the injection. The degree of anesthesia was determined by attempting to elicit corneal, pain and righting reflexes. Anesthesia was considered to exist when the righting and pain reflexes were absent. In the doses used, nembutal caused the disappearance of the corneal reflex only inconsistently.

Nembutal anesthesia lowers the blood pressure of chronically hypertensive rats (expt. 1) but does not affect that of rats recently made hypertensive (expt. 2) or of normal rats (expt. 3).

Nembutal without anesthesia. To control any effect of nembutal other than its anesthetic effects, animals were treated with non-anesthetic doses of nembutal (20 mgm. per kilo), or with full anesthetic doses of nembutal (40 mgm. per kilo) after fortification of the animal against the anesthetic effect by the administration of the central nervous stimulant picrotoxin. The most satisfactory proportions and methods of administration of the nembutal-picrotoxin combination were

as follows: 3.0 mgm. of picrotoxin per kilo were injected intraperitoneally. As soon as intermittent convulsions had begun (about 10 min.), the blood pressure was taken between convulsions, and the animals were reinjected with 1.5 mgm. picrotoxin and 40 mgm. nembutal per kilo. After 15 minutes the stage of anesthesia was determined and the blood pressure taken again. Even with these proportions anesthesia was prevented in only half the animals used, and only these are considered in this report. Animals which yielded to anesthesia responded just as those anesthetized without picrotoxin, but these are not included in the data. Larger doses of picrotoxin were not used as the convulsions were often fatal.

TABLE 1

The response of the systolic pressure of normal rats and of early and late hypertensives to nembutal, picrotoxin and yohimbine

EXPERIMENT NO.	TREATMENT DOSES MGM./KILO	TYPE OF ANIMAL	REMARKS	MEAN BEFORE	MEAN AFTER	DIFFERENCE OF MEANS	RANGE OF INDIVIDUAL DIFFERENCE	NUMBER OF ANIMALS
1	40 N	L	Anesthetized	169	115	−54	−104 to −22	24
2	40 N	E	Anesthetized	151	145	−6	−24 to +43	25
3	40 N	C	Anesthetized	119	113	−6	−23 to +14	12
4	20 N	L	Non-anesthetic dose	175	163	−12	−23 to +1	5
5	40 N	L	Resistant to anesthesia	176	160	−16	−18 to −6	5
6	3 P	C	Convulsions	123	127	+4	−8 to +11	7
7	4.5 P + 40 N	C	Quiet but all reflexes present	127	120	−7	−12 to +4	7
8	3 P	L	Convulsions	168	177	+9	+3 to +15	9
9	4.5 P + 40 N	L	Quiet but all reflexes present	177	170	−7	−20 to −1	9
10	3 Y	L		161	135	−26	−45 to −13	20
11	3 Y	C		120	127	+7	−1 to +15	12
12	3 Y	E		152	153	+1	−22 to +11	12

Col. 2: N, nembutal; P, picrotoxin; Y, yohimbine hydrochloride.

Col. 3: C, normal control animals; E, early hypertensives (less than two weeks); L, late hypertensives (more than two months).

In some cases full anesthetic doses of nembutal failed to produce anesthesia in hypertensive rats for unknown reasons, and the results on these animals are reported separately (expt. 5).

Nembutal does not lower the blood pressure of hypertensive rats even of long standing, unless it causes anesthesia. Experiment 4 shows the effect of small doses, experiment 5 the effect of full doses in anesthesia-resistant rats. The effects of picrotoxin in counter-anesthetic doses in preventing the lowering of blood pressure which nembutal produces in chronically hypertensive rats (exp. 9) cannot be due to a direct pressor action, for it is not pressor in normal animals (expt. 6) and only slightly so in chronically hypertensive animals (expt. 8). The results of experiment 7 indicate that the picrotoxin-nembutal combination is without important effect on the blood pressure of normal rats.

Yohimbine hydrochloride. Yohimbine was injected intraperitoneally as the hydrochloride at a dose of 3.0 mgm. per kilo in several experiments. Yohimbine does not affect the rise in blood pressure caused by angiotonin (24), the active agent in the renal pressor system. On the other hand the sympatholytic and adrenolytic actions of the drug appear to have been proved (25–28). Solutions of yohimbine hydrochloride (Merck) were made up just before use, as the drug is unstable in solution. Blood pressures were taken immediately before and 30 to 60 minutes after the injection. In a preliminary investigation we found that the maximum blood pressure effect of yohimbine in the hypertensive rat was exerted at this time, although some effect persists for 4 hours. *Yohimbine hydrochloride lowers the blood pressure of hypertensive rats of long standing (expt. 10); the blood pressure of recently hypertensive animals is unaffected by the drug (expt. 11); and the blood pressure of normal rats shows a slight elevation (expt. 12).*

Discussion. There is great significance in the fact that two such different substances as nembutal and yohimbine should both serve to distinguish between new and long-standing renal hypertension.

The similar values for blood pressure obtained by Schroeder (29) using nembutal anesthesia and Williams, Harrison and Grollman (23) using conscious animals, indicate that nembutal anesthesia does not influence the blood pressure of normal rats. Our results are in accord with these findings: Schroeder (29) reported hypertensive blood pressure levels in recently hypertensive rats under nembutal anesthesia, but most values were obtained on animals hypertensive for periods less than 40 days. This supports our observation that early hypertension is unaffected by nembutal anesthesia. Page (30) has concluded that the blood pressure of hypertensive dogs (duration not stated) is altered unpredictably by anesthesia. It is our belief that the variability in this response is related to the duration of hypertension. Dock (8) has likewise observed a fall in the blood pressure of hypertensive rabbits after urethane anesthesia, but the duration of the hypertension is not stated.

Three hypotheses suggest themselves as possible explanations of the effects of nembutal anesthesia on the blood pressure of rats with long-standing renal hypertension. The effect may be exerted on 1, some part of a purely humoral mechanism; 2, a neurological mechanism modifying the effect of the humoral mechanism; 3, a purely neurogenic mechanism which may be solely or largely responsible for maintaining hypertension initiated by the humoral renal pressor system.

1. Does nembutal anesthesia affect the secretion, chemical composition or action of the renal pressor substance? Assuming that the renin—renin substrate—angiotonin system as currently conceived is the only humoral system involved, the answer must be "no" since the secretion of renin and response to angiotonin occur in the nembutalized animal after partial occlusion of the renal artery (14) and hemorrhage (31); moreover, the sensitivity of normal and nembutalized animals to renin and angiotonin is the same (30). Furthermore, as just shown, nembutal in the absence of anesthesia does not lower the blood pressure of hypertensive rats of long standing.

2. Does nembutal anesthesia depress a nervous mechanism which maintains

normal vascular responses to the humoral agents responsible for renal hypertension? Again the answer must be "no," in view of the above mentioned equal responses of normal and nembutalized animals to renin and angiotonin. Further evidence is the fact that (skillful) pithing actually increases the response to angiotonin (32). Also the blood pressure of rats in early hypertension is unaffected by nembutal anesthesia (table 1, Experiment 2).

3. There remains the third possibility that nembutal lowers the blood pressure of hypertensive rats of long standing by depressing a purely nervous mechanism involved in its maintenance. The effects of yohimbine lend support to this view. If a nervous mechanism is involved in the maintenance of long-standing hypertension, then its efferent pathway is presumably the sympathetic nervous system, and the sympatholytic action of yohimbine should cause a lowering of blood pressure.

Unfortunately, the action of yohimbine is complicated by the fact that it is also a central stimulant and has a pressor effect in unanesthetized animals (33). Thus the final effect of yohimbine will depend on the balance between its pressor and depressor effects.

In the normal rat the pressor effect predominates, and so sympathetic tone may be presumed to be low. On the other hand, there is a marked depressor effect in the hypertensive animal of long standing, a fact which indicates that sympathetic tone is high. The lack of effect on recently hypertensive rats indicates that the greatest part of the hypertension is maintained by a non-sympathetic (renal?) mechanism, although some augmentation of sympathetic tone may already have occurred (failure of yohimbine to exert a pressor effect).

The findings of Gregory, Lindley and Levene (34) that spinal anesthesia lowers the blood pressure of human beings with essential hypertension to the same level as that of normals suggest that human essential hypertension is neurogenic rather than nephrogenic, and that it is more closely related to the hypertension which we have observed in our renally hypertensive rats of long standing than to early hypertension of renal origin in animals.

Conclusions. Hypertension, precipitated in the rat by interference with the arterial circulation to the kidney, results from a disturbance in the renal pressor system. This mechanism maintains hypertension for some period during which neurocirculatory alterations leading to a new "set" for vasomotor tone occur.

Removal of the precipitating factor at this point (nephrectomy, atrophy of the affected kidney, or development of accessory circulation) does not affect the hypertension which is now maintained by a nervous mechanism.

SUMMARY

Sixty rats made hypertensive by partial ligation of one renal artery and thirty normal rats were subjected in twelve experiments summarized in table 1 to varying doses of nembutal, nembutal and picrotoxin, and yohimbine. All pressure measurements were made with the tail plethysmograph.

Nembutal anesthesia does not affect the blood pressure of normal or recently

hypertensive rats, but lowers the blood pressure of hypertensive rats of long standing.

Nembutal does not lower the blood pressure of any hypertensive rats, even those of long standing, except when it produces anesthesia.

Yohimbine hydrochloride, a sympatholytic agent, lowers the blood pressure of hypertensive rats of long standing, but does not affect the blood pressure of recently hypertensive rats, and may cause a slight elevation in the blood pressure of normal rats. New and old-established experimental renal hypertension may be clearly differentiated by these pharmacological tests.

It is suggested that the renal pressor mechanism which initiates experimental renal hypertension is later superseded by a neurogenic mechanism mediated through the sympathetic nervous system.

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PLASMA, GELATIN AND SALINE THERAPY IN EXPERIMENTAL WOUND SHOCK[1]

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Received for publication April 14, 1944

A 5 per cent solution of ossein gelatin in 0.9 per cent saline serves as an efficient plasma substitute in certain types of shock characterized primarily by loss of blood plasma. For example, gelatin is highly effective in preventing the shock which follows release of tight limb tourniquets, providing it is administered as a series of small intermittent infusions over a period of hours. Likewise it is efficacious in maintaining the circulation and preventing the onset of shock when the Duncan-Blalock leg press is removed (1, 2, 3). In the shock which follows both of these procedures, severe plasma loss into the area of injury is apparently the primary cause of the low blood pressure and eventual circulatory failure.

However, one type of shock studied in this laboratory, i.e., that induced by traumatizing the thigh muscles with numerous blows of a light rawhide mallet, was found to be no more responsive to gelatin infusions than to equal quantities of the saline in which the gelatin is dissolved (1). Shock resulting from this type of limb trauma differs from that following release of tourniquets or a leg press in that whole blood and not plasma alone is lost into the injured area. A nervous factor is also present and seems to play an important rôle (4). In view of the difference in responsiveness to gelatin infusions exhibited by animals shocked by these various procedures, it was decided to study further the effect of plasma substitutes on shock induced by a combination of the two factors: whole blood loss and severe muscle injury.

The procedure finally adopted for producing shock was by gun shot injury to muscle masses of the hind limbs of deeply anesthetized dogs. A uniform and effective method was eventually devised which almost invariably induced fatal shock in untreated animals; however, the shock was reversible in that it would readily respond to appropriate therapeutic measures. The simplicity of the procedure and uniformity of results obtained led us to believe that the method might be of interest to students of the shock problem, and for this reason it will be described in detail.

A Winchester model 67, single shot, smooth bore 0.22 long rifle was employed. This type of gun is especially used for miniature trap and skeet shooting. The shells, each containing 120 to 125 fine lead shot, are manufactured by the Remington Arms Company of Bridgeport, Connecticut. Each of the lead pellets measures approximately 1.2 mm. in diameter. It is essential, if uniform results

[1] We wish to acknowledge our indebtedness to the Upjohn Company of Kalamazoo, Michigan, for providing funds to defray a part of the expenses of this investigation and for generous supplies of specially prepared gelatin.

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are to be obtained, that *only 0.22 long range, high speed shot shells be used.* Shells merely designated as 0.22 long shot shells proved unsatisfactory for producing shock owing to lack of penetrating power. According to the manufacturer, the long range high speed 0.22's develop 25 to 30 per cent higher speed and 50 to 80 per cent greater power than the ordinary 0.22 long shot shell. It is necessary to obtain permission of the War Production Board to buy the shells. Both gun and shells were purchased through Abercrombie and Fitch, Dealers, 45th Street and Madison Avenue, New York City.

Three shots, containing on the average 375 fine lead pellets, were fired into the muscles of the outer surface of each thigh of the deeply anesthetized dog, with the target at a distance of 7 to 8 feet from the gun muzzle. The shots were so placed as to cover from all angles the entire surface of the upper one-third of the limb. Since the area of "scatter" of the small shot at the distance used is approximately 3 inches, overlapping of the area injured by each shot necessarily occurs.

There was always some, and occasionally severe, bleeding accompanied by rapid swelling of the limb first injured. The remaining leg usually exhibited little or no external hemorrhage and the swelling was not so evident. The injured areas were lightly tamped and swathed in cotton in order to decrease bleeding, but in no case were bandages or tape applied.

Blood pressure determinations were made immediately after injury and repeated at various periods thereafter. The first infusions of plasma, gelatin or saline were started within a few minutes after the blood pressure and blood samples were taken. No water was given until 24 hours after injury. At the end of this interval, the surviving animals were allowed water *ad libitum* and offered food.

The animals employed were healthy, vigorous mongrels which had been kept under laboratory conditions for at least one week before using. They were anesthetized by an intravenous injection of 30 mgm. of pentobarbital sodium per kilogram of body weight, and kept under anesthesia for 18 to 24 hours or until death occurred. At intervals throughout this period additional small doses of the anesthetic were administered by intraperitoneal injection. Only well nourished dogs weighing between 6 and 14 kgm. were used; animals either smaller or larger than these were found to be unsuitable. The muscle masses of the hind limbs of the light weight dogs were too small and the high velocity shot frequently broke the femur. On the other hand, tests made on heavy dogs weighing more than 14 kgm. showed that such animals do not always develop shock. These large dogs have thick, tough skin which serves as an effective barrier to deep penetration of the fine lead pellets.

Preliminary to injury, the hair was closely clipped with electric shears, and the limbs thoroughly bathed with soap and water and rinsed with 70 per cent alcohol. The anesthetized animals were then placed on their side in cradles, and the limb held in a vertical position against a back stop of 0.5 inch pine board. Following injury, the animals were left to lie on their side in the cradles in a

suitably warmed room where the temperature was maintained between 22° and 24°C.

The muscles of the inner surface of the limbs should not be used since here the large blood vessels are more superficial and the numerous fine shot cause considerable external hemorrhage. Thus of 11 deeply anesthetized animals tested, using 2 shots on the outer and 1 on the inner surface of each leg, all died in shock with an average survival of 6 hours. However, the femur was found to be fractured in 5 of the animals and severe external bleeding occurred. Since bone injury was regarded as an unnecessary complication this method of placing the shot was abandoned. None of these animals are included with the data discussed in this paper.

In other preliminary experiments, using large and small dogs, and varying the number of shots fired from different ranges, it was found that the procedure described, i.e. 3 shots per leg, and all on the outside and at a distance of 7 to 8 feet from the target, was the most suitable single method for inducing shock and therefore was adopted for all of the experiments.

The literature concerned with gelatin and saline as plasma substitutes in experimental shock induced by various procedures has been referred to in earlier papers by the writers (1, 2) and will not be again reviewed. Likewise adequate reference to the methods employed for blood pressure determinations, hematocrit, hemoglobin and serum proteins have been discussed in these previous communications.

1. *Control series. Untreated animals.* The control group consisted of 43 dogs of which 39 or 90.7 per cent died, and 4, or 9.3 per cent did not exhibit shock symptoms. The average survival period of the animals which died was 11 hours. The interval between injury and death in the series of 39 controls which died varied from 3 to 24 hours. Actually but 2 of the control series survived indefinitely for 1 died 65 hours, and one 42 hours, following injury. The immediate cause of death of these two long surviving cases was obscure but apparently they did not die of shock. Both had normal or near normal blood pressures and had taken food some hours before death. However, in the control experiments any animal surviving 30 hours from the time of injury was counted as a survivor.

A. *Arterial pressure.* The average mean arterial pressure of the 43 animals was 110 mm. Hg. The pressure was determined in the femoral artery by the intra-arterial needle puncture method (5) while the dog was under pentobarbital sodium anesthesia. The range was from 96 to 135 mm. Hg. However, immediately following injury the pressure dropped to an average of 54 mm. Hg in the animals which died, with a range from 28 to 110 mm. Hg. The pressure of the 4 animals which failed to develop shock averaged 93 mm. Hg after injury. These dogs were the maximum size used and autopsy showed that the thick, tough skin had prevented good penetration of the shot. In those animals in which the blood pressure fell to low levels, i.e., 25 to 40 mm. Hg, a slow recovery to 50 to 70 mm Hg occurred in the hours subsequent to injury followed by a later, progressive, secondary decline to levels incompatible with life. Figure 1. Two to 5 hours

after injury the average pressure of the series of 39 control dogs which died was 67 mm. Hg and 7 to 10 hours after injury the average pressure was 57 mm. Hg. The sudden steep fall in arterial pressure immediately following injury, is presumably due to rupture and injury of numerous vessels of the limbs and consequent severe hemorrhage.

B. *Hemoconcentration.* Blood concentration after injury, as measured by hematocrit, hemoglobin and serum protein determinations, was not marked at any time in the control series, although the hematocrit rose slightly (Table 1, fig. 1). This was most evident 2 to 5 hours after injury and was followed by dilution by the 7th to the 10th hour in those animals surviving this interval.

C. *Plasma volume changes.* A series of 7 dogs of the control group was studied for changes in plasma volume after injury. The dye T-1824 was employed and the spectrophotometric method of Gregersen and Stewart (6) was used for the

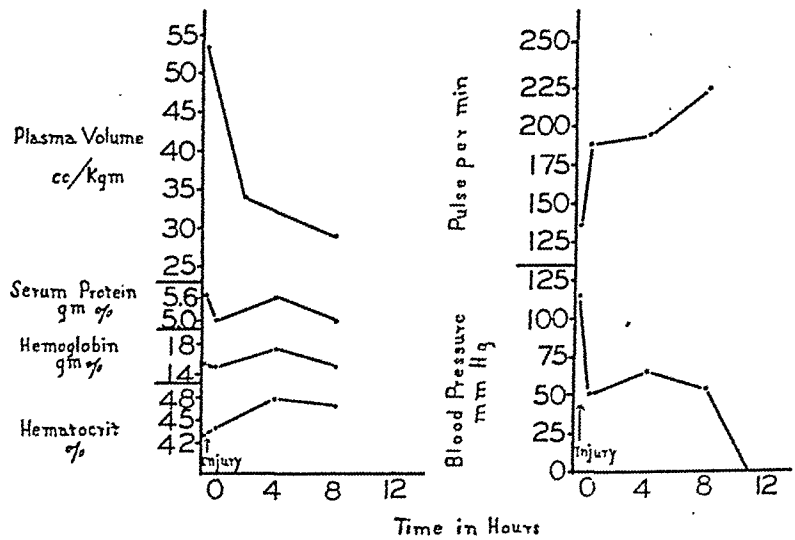

Fig. 1. Average blood pressure, blood volume and hemoconcentration changes in untreated animals subjected to experimental wound shock.

determinations. The average plasma volume of the 7 control animals previous to injury was 52.9 cc. per kilogram of body weight. Four to 7 hours after injury the plasma volume had decreased to an average of 29.7 cc. per kilogram. This represents an average decline of 44 per cent during this interval. Not all of the decrease in plasma volume can be accounted for by loss into the tissues of the limbs because varying amounts escape at the time of injury by external bleeding. Thus the markedly decreased blood volume is due to both external and internal hemorrhage at the site of injury. There was no evidence for loss of fluid elsewhere in the body. Figure 1.

D. *Autopsy findings.* Aside from the bleeding and numerous perforations in the skin and thigh muscles, there were no striking or consistent pathological changes. Gross examination revealed merely pale and anemic organs and tissues characteristic of animals dying of exsanguination.

The limbs exhibited much swelling, and when cut were found to be full of

clotted and extravasated blood, pooled around the broken vessels. The muscles contained numerous lead pellets, and although torn in places showed surprisingly little mutilation despite the numerous small perforations. Very few of the

TABLE 1

Average blood pressure and hemoconcentration changes in experimental wound shock and the effects of plasma, gelatin and saline therapy

	NUMBER OF DOGS	BODY WEIGHT	NO. SURVIVED	NO. DIED	INITIAL (ANESTHESIA)		IMMEDIATELY AFTER INJURY		7–10 HRS. AFTER INJURY		20–24 HRS. AFTER INJURY		48–72 HRS. AFTER INJURY		SURVIVAL
					B.P.	Pulse	B.P.	Pulse	B. P.	Pulse	B. P.	Pulse	B. P.	Pulse	
Untreated control	43	13.7 10.6	4	39	109 110	141 139	93 54	139 189	100 57	164 221					Indef. 11 hrs.
Saline intermittent infusion	23	10.16 10.67	11	12	117 118	134 149	58 50	147 148	94 76	149 156	83	148	97	115	Indef. 14 hrs.
Gelatin intermittent infusion	26	11.44 10.44	16	10	107 99	148 142	69 53	142 150	98 77	142 176	87 52	158 198	107	139	Indef. 20 hrs.
Plasma intermittent transfusion	15	11.74 9.04	11	4	105 110	129 128	66 49	155 169	97 70	173 206	101	158			Indef. 18 hrs.
Plasma single transfusion	14	9.79	2	12	109	146	50	164	39	202					Indef. 14 hrs.
Saline single infusion	10	11.45 8.51	3	7	148 112	144 149	55 57	132 166	97 71	196 186	80	166			Indef. 16 hrs.
					Hemat. %	Hb. grams %	Hemat. %	Hb. grams %	Hemat. %	Hb. grams %	Hemat. %	Hb. grams %	Hemat. %	Hb. grams %	
Untreated control	43	13.7 10.6	4	39	43.5	15.2	43.8	15.1	47.3	14.8					Indef. 11 hrs.
Saline intermittent infusion	23	10.16 10.67	11	12	36.0 42.3	11.6 13.4	41.1 49.0	14.2 15.0	44.9 41.8	14.3 13.3	39.6	12.4	32.8	15.5	Indef. 14 hrs.
Gelatin intermittent infusion	26	11.44 10.44	16	10	39.4 41.0	13.3 14.4	46.9 49.2	13.4 17.9	38.8 36.6	12.4 13.8	42.7 38.3	13.9 14.0			Indef. 20 hrs.
Plasma intermittent transfusion	15	11.74 9.04	11	4	41.3 44.9	15.8 16.9			37.3 42.2	13.3 11.7	37.4	12.2			Indef. 18 hrs.
Plasma single transfusion	14	9.79	2	12	45.3	17.4	42.5	15.4	26.0	13.2					Indef. 14 hrs.
Saline single infusion	10	11.45 8.51	3	7	43.5 49.8	16.9 17.5	36.5 47.8	15.3 16.0	48.3 50.5	18.0 18.3	43.3	17.1			Indef. 16 hrs.

small shot penetrated the limb with sufficient force to pass through the skin of the inner surface to the exterior.

Many of the animals which received plasma transfusions, or infusions of gelatin or saline, either did not develop shock or else recovered. The limbs remained

swollen for a week or more, but were not paralyzed, and the animals moved about without difficulty or evidence of discomfort. By keeping the skin of the injured area clean and covered with sulfonamid ointment, the small skin perforations healed quickly without infection. Some necrosis and sloughing generally appeared after a few days but seemed not to interfere with the health, vigor or appetite of the animals.

2. *The effect of plasma, gelatin and saline therapy.* Any shock inducing procedure to be standardized and easily reproducible should *a*, terminate fatally in nearly all untreated animals within 4 to 24 hours, and *b*, be reversible or preventable and hence responsive to therapeutic or prophylactic measures. The method employed by us appears adequately to meet the first requirement, since 90.7 per cent of 43 untreated controls died of circulatory failure with an average survival period of 11 hours following injury.

In order to test whether shock produced by gun shot injury of the limbs could be prevented or reversed after onset, plasma and the plasma substitutes, gelatin and saline, were infused as therapeutic measures. The infusions were of the intermittent type unless otherwise stated. The amount of plasma or substitutes administered was 33 cc. per kilogram of body weight, given as 5 injections of 6.6 cc. each, distributed over a 7 hour interval. The intravenous infusions were given at 0, 1, 2, 4 and 7 hours after injury.

A. *Intermittent transfusions of plasma.* This series consisted of 15 animals which received pooled heparinized plasma. Of the 15 dogs so treated, 11 or 73.2 per cent did not develop shock and lived indefinitely, and 4 or 26.8 per cent died. The average survival period of the animals that succumbed in shock was 18 hours. This represents an average survival of 7 hours longer than untreated controls. The pertinent data for the group are given in table 1.

It is evident that plasma administered intermittently in small amounts over a 7 hour period is highly efficacious in preventing shock. In these experiments plasma proved to be superior to either gelatin or saline as a therapeutic agent. Not only did a greater per cent of the animals survive, but at the end of 18 to 24 hours when anesthesia was discontinued, the animals appeared stronger and more vigorous than those surviving as a result of either gelatin or saline treatment.

B. *Single massive transfusion of plasma.* For comparison with the intermittent transfusions, a series of 14 animals was given a single large transfusion of 33 cc. per kilogram of pooled, heparinized plasma, immediately following injury. The plasma was injected through the jugular vein, and administered at approximately 10 cc. per minute to 9 dogs, and 15 cc. per minute to the remaining 5 animals. The essential data are given in table 1. Of the 14 dogs used, 12 died and 2 lived indefinitely. It will be recalled that when an equal quantity of plasma was given intermittently as 5 small injections over a 7 hour period, 73.2 per cent survived and 26.8 per cent died. Thus it is obvious that a single massive transfusion given within the first 10 minutes following injury of the type used in these experiments exerts little effect in preventing shock. This was an unexpected finding, for we anticipated that more of the animals would survive as a result of the transfusion.

The greater effectiveness of the small intermittent infusions over single massive infusions presumably is due to greater retention of plasma within the circulation. The volume given at each intermittent injection is not sufficient appreciably to raise the pressure or force the plasma through the injured vessels into the tissues at the site of injury. On the other hand, the plasma given as a single massive infusion rapidly leaks into the tissues of the limbs. The legs of the dogs receiving the single infusion may exhibit marked swelling during the injection, and profuse bleeding through the skin perforations usually started anew.

It has been our experience, insofar as experimental shock in dogs is concerned, that single massive transfusions of plasma are a somewhat wasteful and relatively ineffective procedure compared with the administration of small intermittent injections given over a period of hours. This has proven to be the case in experimental shock induced by release of leg tourniquets, crush injury caused by a leg press, and shell shot wounds of the limb muscles.

In contrast to the animals of the other experimental series, the dogs receiving the single large transfusion of plasma exhibited marked decline in hematocrit and hemoglobin concentrations. The hematocrit fell from the initial reading of 45.3 per cent to 32.0 per cent 7 to 10 hours after injury when the animals were moribund, and the hemoglobin dropped from 17.4 grams per cent to 13.2 grams per cent during the same interval.

C. *Intermittent gelatin infusions.* The material used in these experiments was a 5 per cent solution of bone collagen gelatin in 0.9 per cent saline. It was obtained in sterile containers ready for use, through the courtesy of the Upjohn Company of Kalamazoo, Michigan. A brief description of the properties of this gelatin as specially prepared by this Company for experimental study has been given in earlier communications (1, 2). The oncotic pressure of the material is approximately 70 mm. Hg after sterilization. We have administered this gelatin intravenously in large doses to several hundred animals but have never observed any ill effects from the injections.

The gelatin infused series consisted of 26 dogs subjected to leg injury, of which 16 or 61.7 per cent did not exhibit shock and lived indefinitely, and 10 or 38.3 per cent died in shock. The average survival period of the animals which died was 20 hours. This represents a 9 hour prolongation of survival over untreated controls. The data indicate that although gelatin as a plasma substitute is more effective in preventing shock than 0.9 per cent saline given in equal amounts and over the same time interval, it is apparently inferior to heparinized plasma. This conclusion is in agreement with that reached by us in earlier studies where gelatin was used as a plasma substitute in the treatment of a type of shock which also involved both whole blood loss and severe muscle injury.

D. *Intermittent saline infusions.* Since the gelatin employed for infusions was a 5 per cent solution in 0.9 per cent saline, it was necessary to test a group of dogs using saline as a control for the gelatin series. The saline was administered intermittently and in the same dosage as the gelatin. Twenty-three dogs were used of which 11, or 47.8 per cent survived, and 12 or 52.2 per cent died. The survival value of saline therapy is quite evident from these experiments since 90.7

per cent of untreated dogs subjected to the same shock inducing procedure died. It was somewhat surprising to find that nearly 50 per cent of the saline treated animals survived. However, the writers (1) reported that a similar per cent of anesthetized, saline injected dogs survive the shock induced by muscle trauma of the type induced by 400 to 800 blows upon the muscles of each thigh with a 200 gram rawhide mallet. It is interesting to note that both types of shock in which saline proved most effective as a shock preventive are characterized by loss of whole blood into the area of injury, and extensive injury to muscle masses. On the other hand, saline infusions proved less efficacious in preventing those types of shock due primarily to loss of plasma, such, for example, as release of tourniquets after a 5 hour constriction of both hind limbs of anesthetized dogs, and crush injury produced by application of the Duncan-Blalock leg press (2, 3).

Saline infusions as a therapeutic measure for the treatment or prevention of shock have not received favorable attention from students of the problem, owing to the fact that any beneficial effects of the injection are transient and seldom sustained. However, the saline has in many cases been administered as a single massive infusion instead of by small intermittent injections given over a period of hours, and has usually been employed to revive the animal already in profound shock, rather than used as a prophylactic to prevent shock from developing.

The use of saline infusions, especially as a prophylactic against shock, requires further investigation. Recent experimental work (7–12) by several groups using saline indicates that it is effective in a certain per cent of cases.

E. *Single infusion of saline.* Since intermittent infusions of saline prevented shock from developing in 47.8 per cent of the animals tested, it was of interest to compare the effectiveness of a single massive saline infusion with the intermittent method of administration. This was done in a series of 10 animals. The saline was a 0.9 per cent solution given within 10 minutes after injury. The total amount was 33 cc. per kilogram injected at the rate of 10 cc. per minute. Of the 10 dogs used, 7 or 70 per cent died, and 3 or 30 per cent survived. The average survival period of those that died was 16 hours. Blood pressure and blood concentration data are given in table 1.

These results are comparable to those obtained with single massive infusions of plasma. Neither plasma nor saline when given as a single injection immediately after injury is as effective in preventing shock as are equal quantities of these same substances given intermittently over a period of 7 hours. Owing to lack of sufficient material the efficacy of a single infusion of gelatin was not studied. However, it is doubtful if it would have proven any better than the plasma or saline.

SUMMARY

1. Plasma, gelatin and saline were tested as therapeutic agents in experimental wound shock involving severe muscle trauma plus external and internal hemorrhage at the site of injury.

2. Of 43 deeply anesthetized control dogs not receiving treatment of any kind, 39 or 90.7 per cent died in shock. The anesthesia was maintained for 18 to 24 hours following injury or until the animals died.

3. Shock induced by the method employed is characterized in general by a lack of marked hemoconcentration, drastic fall in blood pressure and loss of whole blood. The plasma volume declined approximately 44 per cent.

4. Transfusions of plasma given intermittently as 5 injections of 6.6 cc. each over a 7 hour interval prevented shock in 11 of 15 animals. Single massive infusions of plasma given within 10 minutes of injury did not prevent shock in 12 of 14 animals used. Much of the injected plasma was rapidly lost into the injured area.

5. Intermittent infusions of gelatin and 0.9 per cent saline were both effective in preventing shock, although gelatin was somewhat more efficacious. Of 26 gelatin infused dogs, 16 or 61.7 per cent survived indefinitely, whereas of 23 saline treated dogs, 47.8 per cent failed to exhibit shock. A single massive infusion of saline prevented shock in but 3 of a group of 10 dogs.

6. Administering small intermittent plasma, gelatin and saline infusions over a period of hours apparently is a more effective method of preventing shock than giving a single massive infusion immediately following injury.

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CIRCULATORY COLLAPSE FOLLOWING MECHANICAL STIMULATION OF ARTERIES

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Received for publication April 22, 1944

The fact that arterial trauma occasionally may cause syncopal reactions has been previously observed. Bazett and McGlone (1) pointed out that puncture of an arterial wall may elicit intense visceral pain capable of producing "reflex responses in the fainting type". The conclusion was drawn that the pain resulting from deep punctures of muscle, connective tissue and periosteum was due to stimulation of nerve plexuses about small arteries. No observations were recorded on the circulatory responses to this type of stimulation. Lewis (2) also observed the production of a "vasovagal" response to painful stimulation of muscles, deep fascia, periosteum and arterial walls. No reports have been found in the literature concerning observations made on the production of syncope in man from relatively painless arterial punctures.

In the course of some experiments on human subjects while in the sitting position, 15 out of 45 individuals developed circulatory collapse following arterial punctures. Observation of these cases was incidental and the experiments had not been planned with the view of studying this type of reaction. The clinical picture which developed bore a striking resemblance to the vasovagal syncope described by Lewis (3). The high incidence of circulatory collapse encountered may be attributed to the fact that subjects were in the sitting position during the arterial puncture and thus were more subject to the development of symptoms from vasomotor changes.

METHODS. It was made clear to each volunteer that the experiment involved the introduction of a needle into an artery. Each subject expressed confidence that this procedure would not bother him and those who collapsed were surprised at having been so affected. Several requested to be used again, but this was possible in only one case. It was the impression of all observers of the experiment that this group of men was emotionally stable.

In each experiment the subject was seated comfortably with his arms resting on adjustable supports. The procedure to be followed in any particular experiment was carefully explained without making any mention of the possibility that faintness or syncope might occur. The skin over the brachial artery, usually on the left side, was anesthetized with a 2 per cent solution of procaine hydrochloride. The subcutaneous tissues and lacertus fibrosis was infiltrated and care was taken to infiltrate well about the artery. A short beveled, 19 gauge needle was introduced into the lumen of the artery and the intra-arterial pressure was recorded by a modified, Hamilton optical manometer (4).

A finger plethysmograph was adjusted to the index finger of the right hand

and connected by capillary rubber tubing to a rubber tambour carrying an eccentrically placed mirror. A calibration syringe for making fine adjustments in the volume of the air in the system was also attached. Recordings were made on sensitized paper run through a camera at the rate of 6 mm. per second.

In two of the experiments the subjects performed a modified Valsalva maneuver, maintaining a column of water at a height of 54 cm. (40 mm. Hg) for 20 seconds.

RESULTS. 1. *Symptoms of collapse.* The symptoms of circulatory failure associated with arterial puncture had an abrupt onset and frequently the entire picture was fully developed within one or two minutes from the time of arterial puncture. Disappearance of pulsations in the artery at the site of the puncture frequently heralded impending collapse.

The symptoms were those of typical syncope: epigastric uneasiness, pallor, sweating, weakness, faintness, dimming of vision, complete loss of vision, and loss of consciousness. Pallor, sweating, weakness and faintness were present

TABLE 1

Incidence of symptoms in ten cases of circulatory collapse

SYMPTOMS	SUBJECT NO.									
	1	2	3	4	5	6	7	8	9	10
Epigastric symptoms	+	−	−	−	+	+	−	+	+	+
Pallor	+	+	+	+	+	+	+	+	+	+
Sweating	+	+	+	+	+	+	+	+	+	+
Weakness	+	+	+	+	+	+	+	+	+	+
Faintness	+	+	+	+	+	+	+	+	+	+
Dimming of vision	+	+	+	0	0	+	+	0	+	+
Loss of vision	0	0	+	0	0	+	0	0	0	+
Loss of consciousness	0	0	0	0	0	+	0	0	0	+

in all; dimming of vision occurred in 7; complete loss of vision in 3 (table 1). There was unconsciousness for 15 to 30 seconds in two subjects during which time convulsive movements were noted. The pupils were observed to be moderately dilated (4–5 mm.) and sluggish in reaction to light.

2. *Blood pressure.* When the symptoms developed rapidly, spontaneous recovery of the blood pressure likewise was rapid even with subjects retained in the sitting position. However, when circulatory collapse appeared more gradually, spontaneous recovery also was more gradual, requiring 5 to 20 minutes.

Because of local vasoconstriction which frequently occurred at the site of the arterial puncture, it was possible to get continuous blood pressure recordings in only 4 subjects who had syncopal reactions.

The blood pressure fell rapidly to alarmingly low levels in the subjects rapidly developing collapse reactions so that only 7 subjects were retained in the sitting position for more than 4 or 5 minutes. As soon as the reclining position was assumed, the arterial blood pressure returned to within normal limits in 1 to 2

minutes although bradycardia, pallor and sweating frequently persisted for 5 to 20 minutes longer (fig. 1).

Blood pressure determined by means of a sphygmomanometer after the needle was withdrawn in 4 subjects are plotted in figure 1, along with the pulse rate. The protocols of these subjects were as follows:

Subject 10. The procaine infiltration was apparently inadequate because evanescent pain resulted during the insertion of the needle into the artery. Thereafter, mild aching

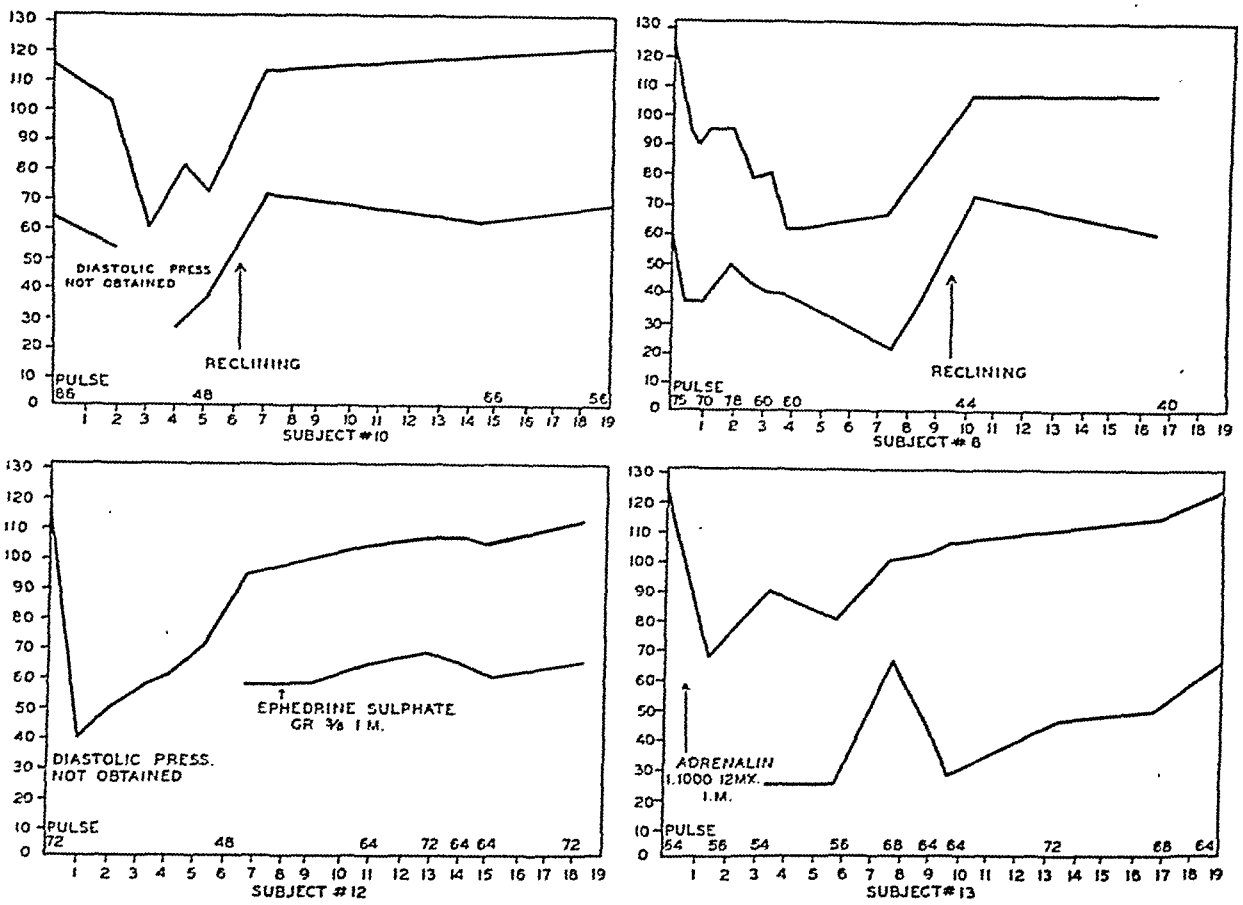

Fig. 1. Graph of systolic and diastolic pressures in 4 subjects. The vertical scale represents millimeters of mercury, the horizontal scale time in minutes. On subject 8 the points shown during the first 4 minutes were obtained from the manometric blood pressure record. All other blood pressures were obtained by the auscultatory method. Pulse rates are recorded along the lower margin of the graph.

persisted about the elbow. The subject complained of dizziness, a sinking feeling in the epigastrium and stated that he was about to faint. In 30 seconds his head fell forward and there were mild convulsive movements. He quickly regained consciousness but was pale, clammy and weak. Flexion of the trunk with lowering of the head made the subject feel better, although his blood pressure remained low until he was placed in the reclining posture. Thereafter, the blood pressure quickly came up to 114/70 mm. of mercury.

Subject 8. This man had collapse in the low pressure chamber at a simulated altitude of 35,000 feet during a classification run two days before the arterial puncture. When he was removed from the chamber his pulse rate was 44 per minute, but the systolic blood pres-

sure was 112 mm. of mercury compared to a control reading of 120 mm. He was placed in the hospital for 24 hours with no further untoward symptoms.

A preliminary blood pressure, determined by means of the sphygmomanometer, was 112/54 while the pulse rate varied from 44 to 48 per minute. An electrocardiogram was taken in view of the bradycardia and found to be normal. Following the routine procaine infiltration, the needle entered the artery painlessly, but a few seconds later the subject became pale and weak. The recording was continued, and during the last portion of it faintness along with tingling of the fingers and of the thumb of the left hand developed. The manometric record of this subject's blood pressure simultaneously recorded with the finger plethysmogram at the onset of collapse and 2 minutes later is shown in figure 5. The pulse rate became rapid and irregular during the first portion of the record, but during the latter part of the 4 minutes of recording it slowed down to 60 per minute and was fairly regular. The heart rate continued to fall after the needle was removed from the artery and was found to be 44 per minute when the subject was placed in the reclining position 6 minutes after arterial puncture had been performed.

The following two experiments were conducted at 20,000 feet. It was planned that the oxygen masks be removed after the completion of the arterial puncture so that the effect of anoxia could be recorded. The appearance of severe vasomotor collapse prevented the completion of the experiment in both cases.

Subject 12. The subject was at 20,000 feet, breathing oxygen with an A8B mask and an A9A regulator set at 35,000 feet. A sudden slowing of the heart rate was noted during the arterial puncture and the needle was quickly withdrawn. The subject stated that he felt empty in the epigastric region. The pulse was not palpable. Within a minute the systolic blood pressure was 40 mm. of mercury but soon rose to 50 mm. The subject was maintained in the sitting position during the descent which required seven minutes and for ten minutes thereafter. At the end of that time he was given ephedrine sulphate 0.024 gram intramuscularly. There was no apparent influence on the blood pressure which had by this time returned nearly to the control level. The duration of the bradycardia seemed to be reduced (fig. 1).

Subject 13. This subject also collapsed during arterial puncture at a simulated altitude of 20,000 feet while breathing 100 per cent oxygen. Immediately after signs of collapse were noted, the needle was withdrawn from the artery and 0.4 cc. of adrenalin (1:1000 solution) was given intramuscularly. About 6 minutes later there was a transient elevation of the diastolic pressure lasting about 3 minutes (fig. 1). No significant bradycardia was noted throughout the experiment although subject was retained in the sitting position.

3. *Plethysmography.* The onset of circulatory collapse coincided with an increase in the volume pulse of the finger, and with bradycardia. In the presence of falling blood pressure the increase of volume pulse indicated the presence of marked peripheral vasodilatation. Figures 2 and 3 show the changes which occurred shortly after mechanical stimulation of the arterial wall. The increase in total finger volume, as shown on the records by a gradual shift of the base line upwards, was noted in almost every case but did not necessarily persist. The total increase in the volume of the index finger was rarely more than 0.25 cc.

4. *Modification of the findings by the Valsalva maneuver.* The effect of increased intrathoracic and intra-abdominal pressures on the circulation during the onset of circulatory failure is shown in figure 4. Subject 5 experienced no symptoms from the Valsalva maneuver during six practice trials prior to the beginning of the experiment. He displayed no pallor or sweating for at least 3 minutes after the arterial puncture was completed. A Valsalva maneuver was then performed

during the recording of the intra-arterial pressure. In contrast with the usual blood pressure response to the Valsalva maneuver, the systolic and diastolic pressures fell progressively during the entire maneuver. At the end of 40 seconds the blood pressure was approximately 64/58 mm. Hg. The pulse rate during the first third of the increased intrapulmonic pressure was 103 per minute. However, during the last two-thirds it fell to 80 beats per minute in spite of a gradual drop in blood pressure. During the last portion of the Valsalva maneuver the subject "blacked-out" completely but did not lose consciousness. The blood pressure failed to reach the initial level following the maneuver, leveling off at approximately 108/50 mm. mercury. The finger volume increased

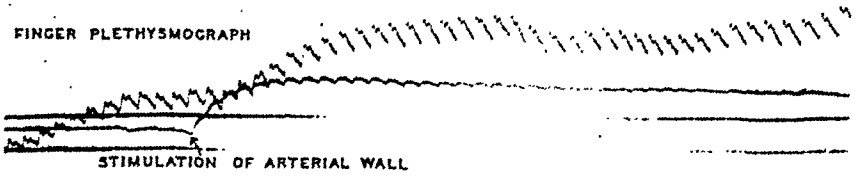

Fig. 2

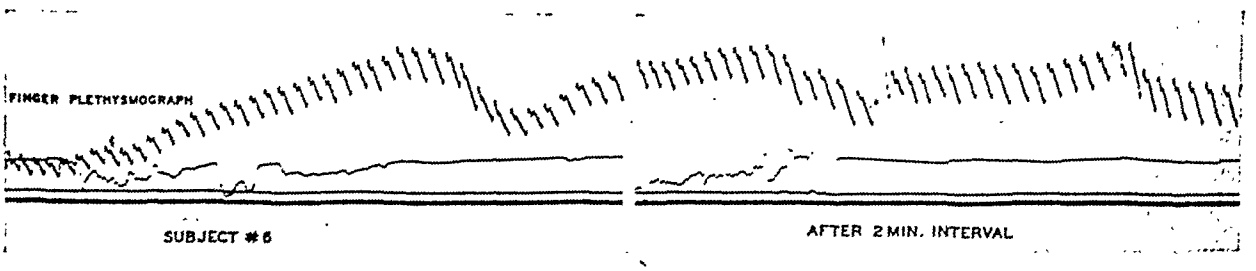

Fig. 3

Figs. 2 and 3. Changes in finger volume, pulse volume and pulse rate following arterial stimulation as recorded by the finger plethysmograph. The record of arterial stimulation was made with the Hamilton optical manometer. In figure 3 the second part is after a 2 min. interval.

much more abruptly than is usually observed during the period of the Valsalva maneuver and fell precipitously at the completion of the maneuver to a level below the original base line.

Subject 14 showed a delayed reaction, becoming suddenly unconscious on standing 2 or 3 minutes after the end of the successful recording of blood pressure during which a Valsalva maneuver was performed (fig. 4). The unusual feature of this record is the relative bradycardia during the last 6 or 7 seconds of the raised intrathoracic pressure and the heights to which the blood pressure rose. The most common change in blood pressure with the Valsalva maneuver is similar to that seen in subject 14 with the exception that tachycardia persists throughout the maneuver and a secondary rise in blood pressure occurring just after the completion of the maneuver is usually more abrupt and higher.

5. *Relation of arterial trauma.* When the arterial puncture was accomplished with minimal mechanical stimulation, in the majority of subjects no collapse

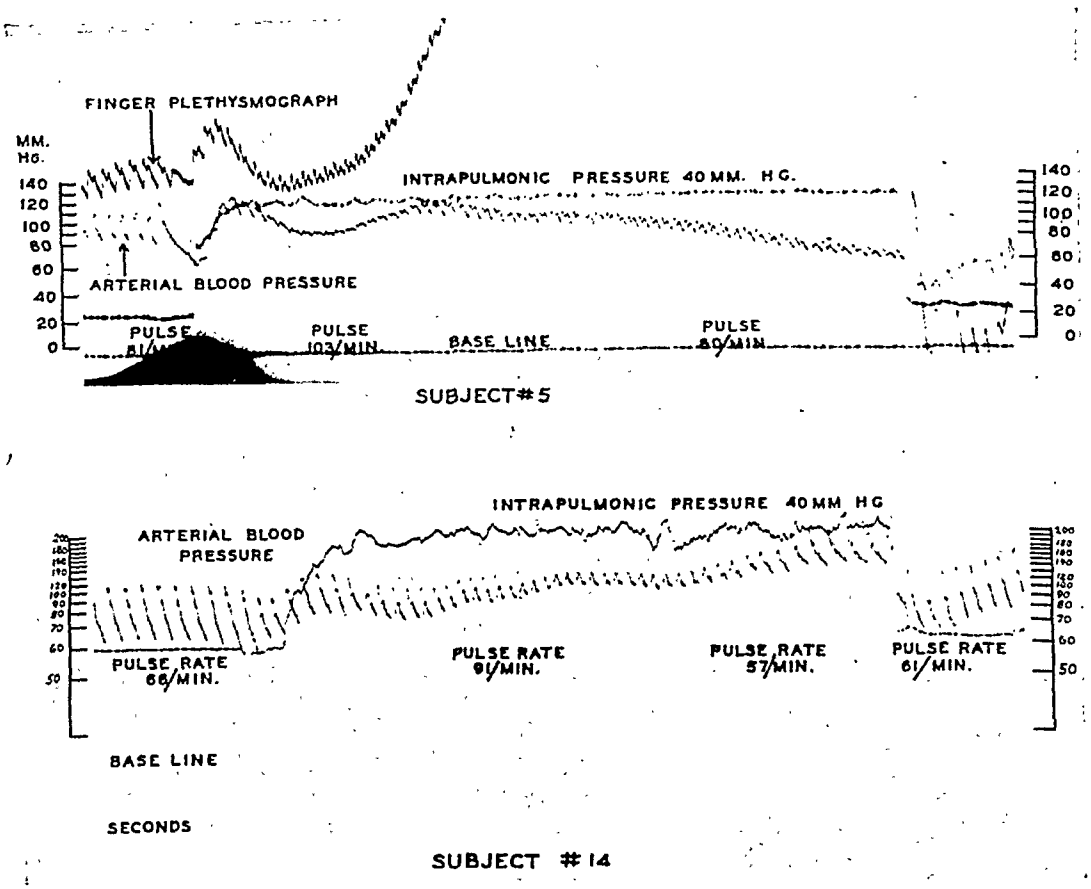

Fig. 4. Intra-arterial blood pressure and finger plethysmograph recordings during Valsalva maneuvers. Subject 5 was in the early stages of collapse and subject 14 suddenly collapsed on standing five minutes after completion of the experiment.

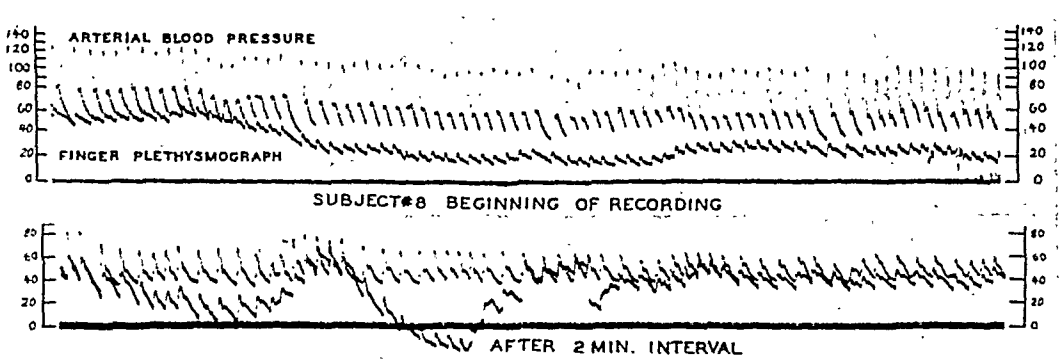

Fig. 5. Continuous intra-arterial blood pressure of a subject gradually developing collapse. There is early tachycardia and progressive fall in blood pressure, pulse pressure and pulse rate during a 6 minute interval.

occurred and the condition of the circulatory system apparently remained normal. In two instances continuous blood pressure recordings from the left brachial artery using a 19 gauge needle with a long bevel were begun without producing

collapse. The brachial artery on the opposite side was stimulated with a short beveled needle of the same gauge. Within a few seconds pulsations disappeared in both brachial arteries and the blood pressure recording failed due to blockage of the needle by the arterial wall.

To study further the psychic factor in the production of the collapse reaction the following experiment was carried out: After careful infiltration of 2 per cent procaine a 19 gauge needle was inserted through the skin and lacertus fibrosis at a point 1 cm. away from the brachial puncture. There were no untoward symptoms for the next 2 or 3 minutes after the needle was withdrawn. It was re-inserted through the skin and impinged upon the artery. The subject did not complain of pain and yet within 30 seconds pallor and sweating were noted, and the subject went on to complete circulatory collapse.

Discussion. It was difficult to evaluate the amount of subjective pain experienced by the various subjects encountered during the arterial punctures. In every case pressure of the needle within the subcutaneous tissues of the arm was felt. Careful interrogation of the subjects revealed that the pain experienced was rarely worse than that found with a common headache and in general resembled a headache in that it was diffuse and poorly localized. In three subjects evanescent sharp pain was noted at the time the needle penetrated the arterial wall. Four subjects stoutly denied that there was any pain during the entire procedure. There was no apparent relationship between the occurrence of collapse and the difficulty encountered in successfully completing the arterial puncture. Several subjects withstood as many as four rather painful attempts at arterial puncture without collapse and others collapsed shortly after the first contact with the artery had been made.

Stimulation of the median nerve was not encountered in any of the subjects in this series. Tingling of the fingers which was noted in a few cases was not limited to the distribution of this nerve and was believed to be due to the local arterial spasm at the site of the arterial puncture.

The possibility that psychic factors played a predominant rôle in the production of this collapse reaction at first was considered quite likely. With continued experience the following factors indicated that this was probably not the case: None of the subjects had a history of fainting from inadequate cause in spite of the fact that all had previously had routine venepunctures. In a number of the cases needles had been placed within veins and left there for several minutes prior to the arterial puncture without producing collapse. A close temporal relation between the arterial puncture and the onset of signs of circulatory collapse was found in almost every case. The fact that pulsations of the brachial artery at the site of arterial puncture disappeared just prior to the beginning of subjective symptoms was frequently observed. All these factors tended to rule out the possibility that psychic factors were the primary cause of the syncopal reaction.

Weiss and his associates (5a, b and c) have exhaustively studied a form of circulatory collapse brought on by the administration of sodium nitrite followed by tilting the subject to 75° on the tilt table. Under these conditions tachy-

cardia appeared while the blood pressure was falling but was superseded by bradycardia at about the time loss of consciousness ensued. They found syncope resulting from this procedure to be associated with findings which pointed to stimulation of both the sympathetic and parasympathetic nervous systems.

Circulatory collapse resulting from mechanical stimulation of an arterial wall closely resembles the response to stimulation of the carotid sinus. Syncopal reactions of this type are fairly common in response to visceral type pain but in this case subjective pain was not a major factor. Gunther et al. (6a, b and c) have studied the venous pressure and intramuscular pressure during shock-like conditions. They have stressed the rôle of a venopressor mechanism in the etiology of these responses. Increase in the volume pulse early in the development of this form of circulatory collapse indicates that peripheral vasodilatation plays an important rôle.

The fact that it was possible to produce circulatory collapse by means of an existing reflex arc without the use of drugs may facilitate investigation of the mechanism and treatment of "primary neurogenic shock".

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SECRETION OF PANCREATIC JUICE AFTER CUTTING THE EXTRINSIC NERVES

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Received for publication April 27, 1944

Very little is known of the possible nervous regulation of pancreatic secretion except that stimulation of the vagus (Pavlov, 1888) or splanchnic nerves (Kudrervezky, 1894) may, under certain conditions, cause secretion of pancreatic juice. The juice collected during stimulation of the vagus nerves contains more than the usual amount of enzymes.[1] Mellanby (1925) advanced the theory that "... the enzyme content of pancreatic juice is determined by the vagus nerves ..." This point of view is also implicit in the writings of Pavlov and his pupils.

Except that a negligible "reflex phase" of pancreatic secretion is known to follow sham feeding in the dog (Walther, 1897), there is no information available regarding the manner in which the nervous mechanism is normally stimulated. Thomas and Crider (1941, 1944) noted that peptone in the intestine caused secretion of very concentrated pancreatic juice like that produced on stimulation of the vagus nerves. Because of this we undertook an investigation of the mechanism through which peptone acts, in the hope of obtaining information concerning the functional innervation of the pancreas. As an initial step we have studied the effect of cutting the extrinsic nerves on the response of the pancreas to peptone in the intestine. We have also looked for any changes that might occur in the secretory effects of soap and HCl.

Ramsay, Thomas and Crider (1943) observed that prolonged stimulation of the pancreas by means of peptone in the intestine caused depletion of the granular material of the acinar cells in normal dogs. The same stimulus administered 24 hours after double vagotomy caused no change in the microscopic appearance of the gland.

METHODS. The methods used for collecting pancreatic juice from dogs and for administering various stimulating substances into the intestine have been described in the publications already referred to. A uniform procedure has been followed in all the experiments. Fasted animals were given measured amounts of the pancreatic stimulus (20 cc. of 5 per cent peptone, 10 cc. of 2 per cent soap solution or 10 cc. of N/10 HCl) into the intestine and the pancreatic juice secreted during the following 10 minutes collected and measured. This procedure was repeated until several successive samples had been obtained. Generally the animal was used for only one experiment in one day and the same stimulus was

[1] The statement, frequently found in text books, that pancreatic juice secreted during stimulation of the vagus nerves contains active trypsin is misleading. Active trypsin is not always present in such secretion and in any case only a fraction of the total proteolytic enzyme secreted is active. (See Babkin, 1928.) According to Guillaumie (1934) this fraction is no greater relative to the total enzyme output than is present in the pancreatic juice secreted in response to secretin under the same conditions.

always used throughout any single experimental period to avoid variations in results due to late effects of previous stimuli.

The first sample collected in any one experiment was seldom typical and was always discarded. The specific gravity of each remaining sample was determined; the samples were then mixed and total nitrogen per cubic centimeter of the mixture determined by the micro Kjeldahl method.[2] In order to obtain an estimate of the effectiveness of each stimulus in promoting the secretion of enzymes under the various experimental conditions, the "total nitrogen per sample" was calculated by multiplying the average volume of the several samples collected during an experiment by the total nitrogen per cubic centimeter. As pointed out previously (Thomas and Crider, 1941), this value is more nearly constant under uniform conditions than either the volume or the nitrogen per cubic centimeter.

The vagus nerves were cut either in the neck or just above the diaphragm. In some animals the vagi were first cut above the diaphragm and later in the neck. Since the second vagotomy did not cause any further change in the response of the pancreas to intestinal stimuli, we consider the intrathoracic operation suitable for this type of study.

The major splanchnic nerves were generally exposed through a flank incision and cut below the diaphragm, though in one animal the left splanchnic was cut above the diaphragm at the time of the vagotomy. Minor splanchnics were also cut whenever they could be found.

RESULTS. *The immediate effects of vagotomy.* In four dogs the vagus nerves were exposed in the neck and a strip of cellophane placed under each and the ends of the strips allowed to protrude from the wound. After the animal had recovered from the operation it was possible to expose the nerves at will by removing the bandage and exerting slight traction on the ends of the cellophane strips. This procedure caused the animal no evident discomfort.

In two of these dogs (4-41, 5-41, table 1), after determining the normal response of the pancreas when the usual quantity of peptone was injected into the intestine, we injected both vagus nerves with cocaine or procaine or cut one nerve and injected the other. Complete vagus block was indicated by acceleration of the heart, usually to about 190 beats per minute, and by absence of the normal respiratory sinus arrhythmia. So long as the nerve block was complete the pancreas failed to secrete significant amounts of pancreatic juice in response to peptone in the intestine even though on several occasions double the usual amount of peptone was administered. The secretory response returned with recovery of the nerves. Recovery was indicated by decreasing heart rate and return of sinus arrhythmia. The absence of secretory response from the pancreas during the vagus block was not caused by the systemic action of the drugs because subcutaneous administration of amounts equal to those injected into the

[2] A study of more than 100 samples revealed the fact that there is a constant linear relation between the total nitrogen and specific gravity of dogs' pancreatic juice. We have taken advantage of this fact in a few cases to supply missing data on total nitrogen. All such instances are indicated in the tables by appropriate symbols.

nerves had no such effect. In one of these dogs the one remaining vagus nerve was cut in the course of an experiment with an effect exactly like cocaine block, i.e., there was no secretory response from the pancreas to peptone for the remainder of that period of observation.

The nerves were cut in the course of an experiment in the other two dogs (6-42, 10-42, table 1). The results were the same as with cocaine block. In one of these one of the nerves was later found to have been incompletely severed but evidently the pancreatic fibers were destroyed. Both of these animals survived for further study and the results of subsequent experiments were comparable in the two animals but only the one (10-42) in which both nerves were completely severed need be considered further. As shown in table 2, there was a partial return of the secretory response of the pancreas to peptone on the day following the vagotomy (expt. 10-29-42) and five days later (11–3–42) still further recovery was evident.

TABLE 1

Immediate effect of cutting or blocking the vagus nerves on the volume and specific gravity of the pancreatic juice secreted in response to peptone in the intestine

DOG NO.	NORMAL			VAGI BLOCKED OR CUT		
	Number of experiments	Average volume	Average sp. g.	Number of experiments	Average volume	Average sp. g.
		cc.			*cc.*	
4-41	2	2.3	1.0158	2*	0.3	
5-41	4	1.9	1.0182	4*	0.65	1.016
6-42	3	2.86	1.0196	1†‡	0.3	
10-42	3	2.24	1.0207	1‡	0.56	1.0142

* Vagi blocked with cocaine or procaine.
† One vagus not completely severed.
‡ Vagi cut in the neck.

The vagus nerves have been cut or blocked in the same way in other dogs (not included in table 1) which had already recovered from previous intrathoracic vagotomy and were again responding to peptone stimulation. In these there was no further change in the pancreatic response to peptone; consequently the results recorded in table 1 cannot be attributed to general systemic disturbances associated with manipulation of the vagi.

Chronic effects of vagotomy. The effect of peptone in the intestine on pancreatic secretion was studied in four dogs that had survived vagotomy for several days or weeks. Control experiments had previously been performed on all but one of them. In three dogs the vagus nerves were cut in the thorax and in one of the three they were subsequently cut in the neck. In the fourth dog the nerves were cut in the neck only. Observations were made at various times, ranging from one day to two and one-half months after operation. All of these dogs secreted significant amounts of pancreatic juice in response to peptone in the intestine after vagotomy. However, both the volume of secretion and the total nitrogen output (nitrogen per sample) remained consistently below normal.

For example, the volume of the 10-minute samples fell from an average normal of 2.27 cc. to 1.52 cc. following vagotomy in the three dogs for which normal data are available. In the same experiments the average total nitrogen per sample decreased from a normal value of 16.07 mgm. to 10.23 mgm. These results are presented in table 2.

TABLE 2

Permanent effect of cutting the vagus nerves on the volume, specific gravity and total nitrogen of the pancreatic juice secreted in response to peptone in the intestine

DOG NO.	NORMAL						VAGI CUT					
	Date	Number of samples	Volume	Sp. g.	Total N	Total N	Date	Number of samples	Volume	Sp. g.	Total N	Total N
			cc.		*mgm./cc.*	*mgm./sample*			*cc.*		*mgm./cc.*	*mgm./sample*
1-42							1-21-42	3	1.6	1.0172	5.76*	9.21*
1-42							1-23-42	3	0.966	1.0200	7.4	7.1
1-42							1-26-42	3	1.0	1.0167	5.44	5.44
9-42	4-23-42	6	1.95	1.0186	6.6	12.87	7- 1-42	3	1.5	1.0187	6.63*	9.94*
9-42	4-28-42	5	2.34	1.0190	6.8	15.91	7- 3-42	1	2.2	1.0205	7.61*	15.22*
9-42							7-10-42‡ a.m.	7	1.54	1.0179	6.37	9.80
9-42							7-10-42‡ p.m.	5	1.09	1.0178	6.02*	6.56*
10-42	7-24-42	5	2.23	1.0220	8.64	19.95	10-29-42‡	4	1.82	1.0171	6.05	11.01
10-42	7-27-42	6	2.16	1.0217	8.11	17.49	11- 3-42‡	5	2.48	1.0179	6.02	14.82
10-42	10-28-42§	4	2.26	1.0185	6.3	14.23						
11-42	7-30-42	6	2.72	1.0152	4.8	13.05	12-28-42†	5	0.68	1.023	9.25	6.29
11-42	7-31-42	6	2.0	1.0189	6.93	13.86	12-30-42†	6	1.06	1.0205	6.93	7.34
11-42	8-14-42	6	2.58	1.0205	7.7*	19.86*	1- 4-43†	3	0.9	1.0213	8.17	7.35
11-42	11- 9-42¶	6	2.3	1.0196	6.96	16.0	1-11-43†	4	0.64	1.0285	13.44	8.60
11-42	11-10-42¶	6	2.15	1.0239	9.45	20.31						

* Nitrogen calculated from sp. g.—594.8 (sp. g.—1.00755) = N in mgm./cc.
† Both splanchnics also cut.
‡ Vagi cut in neck.
§ Vagi exposed but not cut, 10-27-42.
¶ Right vagus cut in neck, 10-27-42.

A noticeable change in latent period was also observed. In the normal animal secretion generally begins in from one to three minutes following the injection of peptone into the intestine. In the vagotomized dogs a single injection of peptone often failed to cause any secretion and occasionally a second injection also was without effect. After secretion was once obtained the latent period following each injection was noticeably longer than in the control experiments; however, exact measurements were not made.

The secretory response to HCl was also studied in three of these animals before

and after vagotomy. Results are presented in table 3. Unfortunately the number of observations following vagotomy is inadequate but from the available data it appears that there was a reduction in the volume of pancreatic juice secreted in response to a standard dose of HCl in the intestine. The total nitrogen per cubic centimeter remained within the range of the lower normal values. Consequently there was a considerable decrease in the total nitrogen per sample.

The secretory response to soap was studied in the same three animals (table 4) In contrast to the results with peptone and HCl, the response to stimulation by means of soap was not affected by vagotomy except in one animal in which the splanchnic nerves were also severed. Since cutting the splanchnics alone did

TABLE 3

Effect of vagotomy on the volume, specific gravity and total nitrogen of pancreatic juice secreted in response to HCl in the intestine

DOG NO.	NORMAL						VAGI CUT					
	Date	Number of samples	Volume	Sp. g.	Total N	Total N	Date	Number of samples	Volume	Sp. g.	Total N	Total N
			cc.		*mgm./cc.*	*mgm./sample*			*cc.*		*mgm./cc.*	*mgm./sample*
9-42	4-30-42	6	7.63	1.0093	1.26	9.61	7-10-42*	5	2.88	1.0092	1.125	3.24
9-42	5-11-42	5	5.64	1.0119	1.82	10.26						
10-42	8-10-42	4	3.37	1.0094	1.0	3.37	10-29-42*	3	5.46	1.0098	0.56	3.05
10-42	8-13-42	4	4.4	1.0086	0.8	3.52	11- 2-42*	4	3.18	1.0086	1.64	5.1
10-42	8-17-42	3	7.43	1.010	1.1	8.17						
10-42	9-26-42	4	3.75	1.0118	2.5	8.62						
11-42	8-12-42	4	7.9	1.0096	0.90	7.11	1- 7-43†	6	2.55	1.0092	1.5	3.8
11-42	8-15-42	3	7.26	1.0096	0.94	6.82						
11-42	8-17-42	3	6.83	1.0096	1.03	7.03						

* Vagi cut in neck.

† Both splanchnics also cut.

not affect the response to soap stimulation, the cause of the peculiar result in this one animal remains obscure.

The effect of cutting the splanchnic nerves. The splanchnic nerves were cut in two dogs. In one (11-42) the vagi were also cut and the results are recorded in tables 2, 3 and 4. The results in this animal did not differ greatly from those observed when the vagi only were cut except that the decrease in volume of secretion following vagotomy was more pronounced and there was, on the average, an increase in the total nitrogen per cubic centimeter so that the decrease in the total nitrogen per sample was less than the decrease in volume of secretion.

In the other dog only the splanchnic nerves were cut. The results are recorded in table 5. There is little evidence of a consistent change in the response to any of the three stimuli following splanchnicotomy in this dog. There appeared to be an increase in the volume of the secretion in some of the experi-

ments, particularly those with soap and HCl, but since this result was opposite to that observed in the other animal it is probably not significant.

TABLE 4

Volume, specific gravity and total nitrogen of pancreatic juice secreted in response to soap in the intestine before and after cutting the vagus nerves

DOG NO.	NORMAL						VAGI CUT					
	Date	Number of samples	Volume	Sp. g.	Total N	Total N	Date	Number of samples	volume	Sp. g.	Total N	Total N
			cc.		*mgm./cc.*	*mgm./sample*			*cc.*		*mgm./cc.*	*mgm./sample*
9-42	5- 5-42	6	4.8	1.0122	2.8	13.44	7-10-42*	2	6.4	1.0106	2.28	14.59
9-42	5- 7-42	5	7.56	1.0120	2.35	17.76	7-11-42*	6	4.7	1.0120	2.64‡	12.40‡
10-42	8-11-42	6	4.8	1.0126	2.8	13.44	10-30-42*	4	4.75	1.0111	2.06	9.78
10-42	8-15-42	5	4.32	1.0124	2.7	11.66	11- 4-42*	4	4.5	1.0144	3.92	17.74
11-42	8-15-42	3	5.76	1.0119	2.4	13.82	1-14-43†	6	1.3	1.0147	5.18	6.72
11-42							1-15-43†	4	1.14	1.0154	5.21	5.93

* Vagi cut in neck.
† Both splanchnics also cut.
‡ Nitrogen calculated.

TABLE 5

Volume, specific gravity and total nitrogen of pancreatic juice secreted in response to various intestinal stimuli before and after cutting the splanchnic nerves with vagi intact. Dog 4-43

NORMAL						SPLANCHNICS CUT					
Date	Number of samples	Volume	Sp. g.	Total N	Total N	Date	Number of samples	Volume	Sp. g.	Total N	Total N
Peptone											
		cc.		*mgm./cc.*	*mgm./sample*			*cc.*		*mgm./cc.*	*mgm./sample*
2-15-43	6	2.25	1.022	8.47	19.05	3-15-43	5	2.54	1.0209	8.71	22.12
3- 2-43	4	2.67	1.026	10.01	26.72	3-19-43	7	2.53	1.0292	11.9	29.10
						3-20-43	5	2.08	1.0187	7.14	14.58
Soap											
2-17-43	4	3.92	1.0178	6.16	24.14	3- 8-43	4	5.9	1.0144	4.34	25.60
2-19-43	5	4.68	1.0153	4.48	20.06	3-16-43	4	6.05	1.0152	4.58	27.70
2-27-43	6	5.92	1.0145	4.27	25.17	3-22-43	4	8.0	1.014	3.22	25.76
HCl											
3- 3-43	5	4.9	1.0088	0.7	3.43	3-20-43	5	8.3	1.0094	1.26	10.45

Discussion. Perhaps the most significant outcome of this study is the observation that section of the extrinsic nerves ultimately has little effect on the

response of the pancreas to intestinal stimuli including some which might reasonably be expected to excite the pancreas through its nerves. Division of the splanchnic nerves was without significant effect and section of the vagi caused, as a permanent effect, only a relatively minor quantitative decrease in the responses to peptone and HCl. The qualitative differences in the pancreatic juice secreted in response to different stimuli (Thomas and Crider, 1944) persisted undiminished after vagotomy.

Nevertheless, the results conform to a pattern which is characteristic of the innervation of some of the other gastro-intestinal viscera. For example, gastric motility returns almost to normal after a temporary depression following section of the vagus nerves (Carlson, 1913). In a discussion of the innervation of the stomach, Carlson pointed out the fact that "... visceral nerves are complex association paths connecting cerebrospinal and visceral reflex centers rather than relatively simple nerves like the skeletal efferents" (Carlson, Boyd and Pearcy, 1922).

The existence of "visceral reflex centers" for the pancreas has not been demonstrated; on the other hand, there is no evidence to support an assumption that the innervation of the pancreas differs fundamentally from that of other gastro-intestinal organs. Our results can, we think, best be explained by the hypothesis that the nervous regulation of pancreatic functions is mediated for the most part through a peripheral reflex mechanism which, in addition to impulses initiated locally, receives impulses from the central nervous system by way of the extrinsic nerves, chiefly the vagi. These impulses may either augment or inhibit local reflexes. The temporary failure of the pancreas to respond to certain stimuli following vagotomy may indicate that some local reflexes occur normally only when facilitated by impulses coming over the vagi; the subsequent recovery may mean that the peripheral mechanism becomes hypersensitive after vagotomy and thus becomes capable of independent function. This conception may also explain many of the peculiarities of the reaction of the pancreas to electrical stimulation of the vagus nerves, e.g., the occasional inhibitory effects, the long latent period, and the occasional unexplained failure of the pancreas to respond.

A simpler hypothesis would be that the vagi are only remotely related to pancreatic function and all the responses of the pancreas, before and after vagotomy, are due to two or more hormones, some of which (e.g., secretin) promote the secretion of water and others (e.g., "pancreozymin", Harper and Raper, 1943) promote the secretion of enzymes. However, this latter hypothesis fails to explain some of our results; consequently we prefer the former.

SUMMARY. 1. Cutting the vagus nerves or blocking them by means of drugs was followed by temporary absence of the secretion of pancreatic juice which normally follows injection of peptone solutions into the intestine.

2. Twenty-four hours or more following vagotomy pancreatic juice was again secreted in response to peptone stimulation but in less than normal amounts. The response to HCl was also less than normal.

3. Vagotomy did not affect the volume or concentration of pancreatic juice secreted in response to soap in the intestine.

4. The total nitrogen content of the pancreatic juice was not consistently altered by vagotomy, regardless of the stimulus.

5. Cutting the major splanchnic nerves caused no consistent change in the response of the pancreas to intestinal stimuli.

CONCLUSION

It is suggested that the vagus nerves influence the external secretory function of the pancreas through augmentation or inhibition of local reflexes.

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